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Recognizing Plant Defense Priming

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Abstract

Defense priming conditions diverse plant species for superinduction of defense, often resulting in enhanced pest and disease resistance and abiotic stress tolerance. Here, we propose a guideline that might assist the plant research community in a consistent assessment of defense priming in plants.

Glossary

Allocation costs are fitness losses caused by allocation of metabolic resources toward defense that would otherwise have been allocated to growth and reproduction.

Defense priming induces a physiological state (the **primed** state of defense) in which a plant is conditioned for the superactivation of defenses to environmental challenges.

Ecological costs occur when fitness-relevant interactions of an organism with its natural environment are impaired.

Memory refers to the processes by which information of an environmental stimulus is stored and maintained for future use.

Naïve state refers to a state of plant or cell, in the absence of stress or stress memory.

Plant fitness is defined as a plant's genetic contribution to the next generation. Seed production, number of flowers, pollen quality and number, and plant growth among others, are generally accepted proxies for plant fitness.

Priming stimulus refers to the trigger that initiates defense priming. The priming stimulus can be a stress itself, an indicative of an imminent stress, a chemical compound, or a beneficial organism. The priming stimulus does not, or only slightly and transiently activate defense responses. It rather promotes the plant to a persistently primed state of enhanced defense readiness.

Transgenerational defense priming refers to the transmission of the primed state from a parental plant to its offspring.

Triggering stimulus refers to an external factor that activates a stress response.

An ecogenomic approach for studying defense priming

Ten years after publication of the seminal review ‘Priming: getting ready for battle’ [1], the importance of **defense priming** (Glossary) as an adaptive trait for the adjustment of plant defense in unpredictable environments is well established. Defense priming has been reported for a wide range of plant taxa, including wild species and cultivated varieties, and from herbaceous to long-lived woody plants [2]. Defense priming is postulated to be an adaptive, low-cost defensive measure because defense responses are not, or only slightly and transiently activated by a given **priming stimulus**. Instead, defense responses are deployed in a faster, stronger and/or more sustained manner following the perception of a later challenging signal (the **triggering stimulus**) **–** that is, in times of stress [2, 3]. Recent studies revealed that defense priming can pass down generations, indicating an epigenetic component in **transgenerational defense priming** [4].

Molecular studies of defense priming recorded changes to chromatin and the accumulation of mRNA of genes with a signaling role in defense, of signaling proteins and pattern-recognition receptors, metabolites, and other molecular components supporting a faster, stronger, and more sustained response to a triggering stress. Because priming is often postulated to improve **plant fitness** in complex environments, the relevance of the molecular findings should ideally be tested in experiments evaluating plant performance and fitness in relevant ecological conditions. However, the ecological investigation of defense priming mostly addressed the impact of a first stimulus on the interaction of plants with other community members, such as microbes, insects and con- and hetero-specific plants. Many of the studies assessed just a few defensive traits, thereby ignoring the overall defensive status of **naïve** vs. **primed** plants (i.e. before exposure to a triggering stress). Moreover, better plant performance under enemy pressure does not necessarily reflect defense priming, because there are additional mechanisms by which plants can adjust their defensive state to the environment. Such additional mechanisms encompass, for example, directly induced defenses, an enhanced tolerance to biotic and abiotic stress, cross-protection from viruses and microbial pathogens, or acclimation. A methodological approach for studying defense priming should, therefore, ideally integrate both molecular analyses of plant defense modulation and the ecological assessment of fitness-related costs and benefits (Figure 1).

Key characteristics of defense priming

Defense priming can be induced by chemical compounds (e.g. β-aminobutyric acid, salicylic acid, pipecolic acid, jasmonic acid, volatile organic compounds), pathogens, insect herbivores, or environmental cues that indicate an increased probability of attack (e.g. insect eggs) [2, 3]. Plant defense can also be primed by beneficial soil organisms such as rhizobacteria and rhizofungi [5]. Depending on the nature of the priming stimulus and the stressor, priming can engage diverse mechanisms [6]. However, irrespective of the inducing and target stimulus, defense priming has characteristic key features. Here, we propose some key criteria that might help in assessing the presence of defense priming in plants (Figure 2).

☑ Memory

When a plant is primed, the information of the priming stimulus is stored, eventually until exposure to a triggering stimulus. We refer to this effect as the **memory** in plant defense [3, 7]. In Arabidopsis, several molecular markers were found to be useful for detecting the primed state. They include elevated levels of pattern-recognition receptors (e.g. FLS2, CERK1), enhanced accumulation of mitogen-activated protein kinases MPK3 and MPK6, augmented expression of transcription factor genes (e.g. WRKYs, MYC2), certain modifications to histones (e.g. trimethylation of lysine residue 4 in histone H3), and DNA hypomethylation [3]. More reliably, but also more elaborately, memory can be revealed by applying at least two sequential incidents: (*i*) the priming event, which primes the defense-related traits, and (*ii*) the challenge (the triggering stress), which activates the defense-related traits at the phenotypic level in a more robust manner in primed as compared to unprimed plants [8] (Figure 1). The time span between the two events is not defined and may vary among stimuli. However, any memory effect would allow some time to pass between the perception of the priming stimulus and the triggering stress. During this time, the defense traits in question, which often are only slightly and transiently induced by the priming stimulus, would return to nearly basal levels. Recent findings revealed that at least some types of defense priming can be inherited, a phenomenon referred to as transgenerational priming. Although the molecular events associated with transgenerational priming remain largely unknown, DNA demethylation has been suggested to contribute to the phenomenon [4]. However, assessing heritability of priming can be challenging, especially when working with non-model and slow-growing plants.

☑ Low fitness costs

Defense priming is expected to cause an overall positive cost-benefit balance in times of stress [9]. Therefore, assessing priming would require an evaluation of the fitness consequences of activating and maintaining the primed state of enhanced defense (i.e. storage of information after priming). Although defense priming has lower costs than the direct activation of defenses, it might still incur some **allocation costs** (and/or **ecological costs**), probably because it causes physiological alterations (e.g. deposition of dormant signaling enzymes, modification to histones on defense gene promoters [3]) while shifting the plant to the alert. However, the fitness-related advantage of priming becomes obvious only upon exposure to a triggering stress, after which primed plants outperform unprimed plants (Figure 1). Thus, the benefits of priming outweigh its costs in hostile conditions [9, 10]. Although this is well appreciated, surprisingly few studies have measured the fitness effects of defense priming. Allocation costs of priming can only be determined in situations that lack the potential benefits of being primed (i.e. before exposure to a triggering stress toward the end of the memory-retaining period). Useful traits for evaluating the fitness costs of defense priming include key physiological processes, such as seed production (see 'plant fitness' in the Glossary).

☑ **More robust defense**

The molecular, biochemical, and physiological events associated with phenotypic defense are faster and/or stronger and/or activated earlier in primed vs. unprimed plants (Figure 1). Primed plants often also display longer-lasting activation, or attenuated repression of defense upon challenge, than unprimed plants [2]. Defensive traits may include, amongst others, changes in defense-related signaling compounds or processes (e.g. hormones and enzymes, alterations to chromatin, enhanced presence of pattern-recognition receptors), or actual defense responses such as accumulation of phytoalexins, glucosinolates, phenolic compounds, reactive oxygen species, lignin, or herbivory-induced plant volatiles [6]. Because naïve plants can be produced in the lab, it is feasible to compare defense responses in naïve-challenged vs. primed-challenged plants. However, such studies are difficult for plants taken from natural habitats or in the field in which naïve plants are essentially unavailable. Nevertheless, field studies have revealed the presence of defense priming in plants at their natural habitat [11].

☑ **Better performance**

Although more robust defense is usually associated with better performance in times of stress, boosting induced defense responses does not necessarily provide an advantage. For example, negative hormonal crosstalk has been reported for induced defenses against herbivores and necrotrophic pathogens on the one hand and biotrophic pathogens on the other. In consequence, an attack by insects sometimes compromises the future capacity of a plant to mount defense against biotrophic pathogens, whereas infection by biotrophic pathogens can affect the plant's ability to mount effective defense against later attack by insects or necrotrophic microbes [12]. These examples emphasize the importance of studying under ecologically realistic conditions whether priming influences plant fitness. It is also important to wonder which plant-response variables are the most appropriate ones for evaluating the benefit of priming. Whereas the contribution of priming to disease and pest resistance can readily be tested by comparing the obvious damage caused by plant enemies or their performance on primed vs. unprimed plants, a role in plant defense implies that the appearance of priming is associated with a gain of plant fitness. Therefore, the impact of priming on plant performance must ideally be demonstrated in terms of plant survival or reproduction. Furthermore, the benefits of defense priming frequently become evident only in ecologically realistic scenarios, in which the plant might experience resource limitation or multiple interactions with other community members.

Further characteristics of defense priming

In Figure 2, we suggest a guideline with some key criteria to test the presence of defense priming in plants. In the text below, we describe additional characteristics that usually are associated with priming. Although informative, the study of such characteristics might be difficult, especially for non-model and slow-growing plants and/or when requiring experiments in natural habitats.

- ☑ **Broad-spectrum activity**

Because defense priming is a state of enhanced defense readiness, which has been associated with enhanced levels of pattern-recognition receptors, priming helps defeat a broad spectrum of diseases and pests [3]. Priming enhances multiple (if not all) defense responses stimulated by a given biotic or abiotic stress and thus also augments the plant’s defense to pathogens, pests, and abiotic stresses related to the priming stimulus. In the absence of a subsequent biotic or abiotic triggering stimulus primed plants usually only marginally activate direct defense responses.

- ☑ **Low ecological costs**

Although the ecological costs of defense priming are expected to be low, strong experimental evidence for this is scarce. Ecological costs can result, for example, from the deterrence of mutualists (predators and parasitoids of herbivores, symbiotic fungi or bacteria, etc.) or reduced intra- or interspecific competitive power. Therefore, the ecological costs of defense priming can only be detected in variable natural habitats with multiple interacting species.

Conclusions

Defense priming is a complex phenomenon that conditions plants for enhanced defense against diverse environmental challenges. Ideally, the presence of defense priming would be supported by a phenotypic analysis of a plant's defensive state before and after later challenge with a biotic or abiotic stress, combined with an assessment of the resulting cost-benefit balance. Such studies are only feasible in multi-factorial experiments [2], including naïve, primed, naïve-and-triggered, and primed-and-triggered plants.

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Reference list

1 Conrath, U. *et al*. (2006) Priming: getting ready for battle. *Mol. Plant Microbe Interact*. 19, 1062-1071.

2 Hilker, M. *et al*. (2015) Priming and memory of stress responses in organisms lacking a nervous system. *Biol. Rev.* http://dx.doi.org/10.1111/brv.12215.

3 Conrath, U. *et al*. (2015) Priming for enhanced defense. *Annu. Rev. Phytopathol.* 53, 97-119.

4 Luna, E. *et al*. (2012) Next-generation systemic acquired resistance. *Plant Physiol.* 158, 844-853.

5 Pieterse, C.M.J. *et al*. (2014) Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347-375.

6 Balmer, A. *et al*. (2015) The 'prime-ome': towards a holistic approach to priming. *Trends Plant Sci.* 20: 443-452.

7 Kandel, E.R. *et al*. (2014) The molecular and systems biology of memory. *Cell* 157, 163-186.

8 Walter, J. *et al*. (2013) Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environ. Exp. Bot.* 94, 3-8.

9 Van Hulten, M. *et al*. (2006) Costs and benefits of priming for defense in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5602-5607.

10 Walters, D.R. *et al*. (2009) Priming for plant defense in barley provides benefits only under high disease pressure. *Physiol. Mol. Plant Path.* 73, 95-100.

11 Heil, M. and Silva Bueno, J.C. (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5467-5472.

12 Lazebnik, J. *et al*. (2014) Phytohormone mediation of interactions between herbivores and plant pathogens. *J. Chem. Ecol.* 40, 730-741.

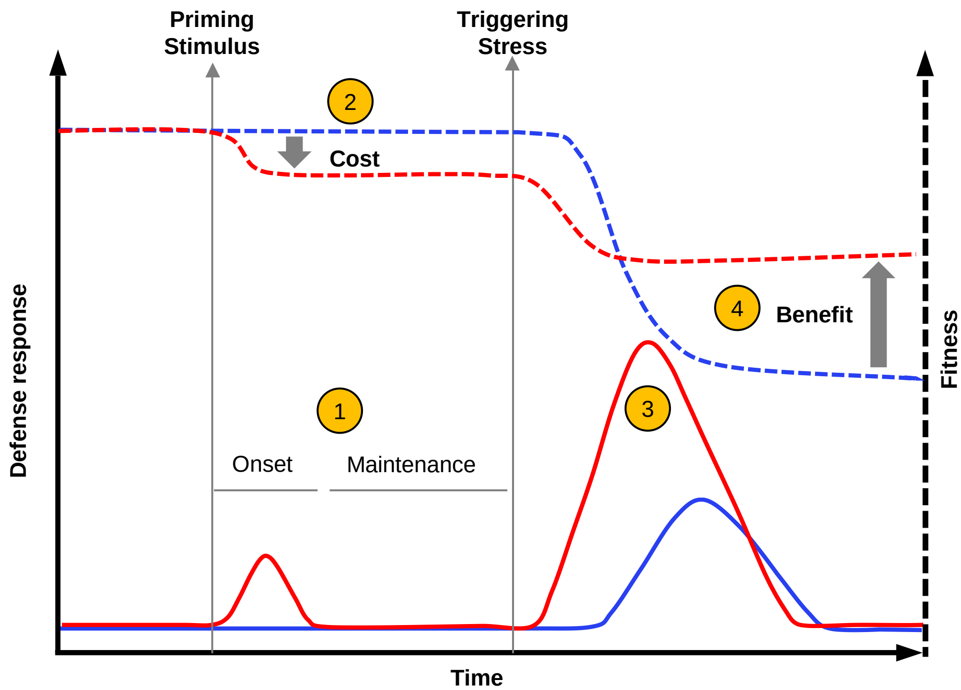

Figure 1. **Scheme of the relationship between defense responses (solid lines) and fitness (dashed lines) in primed (red) vs. unprimed (blue) plants**. Analysis of defense priming requires a set of steps encompassing both the assessment of plant defenses and the associated cost-benefit balance. Here we suggest some criteria that may help in deciding whether defense priming is present:

1. Memory - Two sequential environmental events are required for asserting memory in the absence of molecular markers: the priming stimulus and the triggering stress. During priming and in the primed state (before the triggering stress) plant defenses are expected to be only transiently and generally faintly induced.

2. Low fitness costs - The maintenance of the primed state (before the triggering stress) has low fitness costs as compared to the direct activation of defense.

3. A more robust defense response - In response to the triggering stress, primed plants mobilize cellular defenses in a faster, earlier, stronger, and/or more sustained manner than unprimed plants.

4. Better performance - Primed plants are expected to defend better against a given stressor than unprimed plants. Therefore, priming enhances plant fitness in hostile environments.

The figure is an adaptation from Hilker *et al*. [2], Balmer *et al*. [6], and Jurriaan Ton's WordPress.

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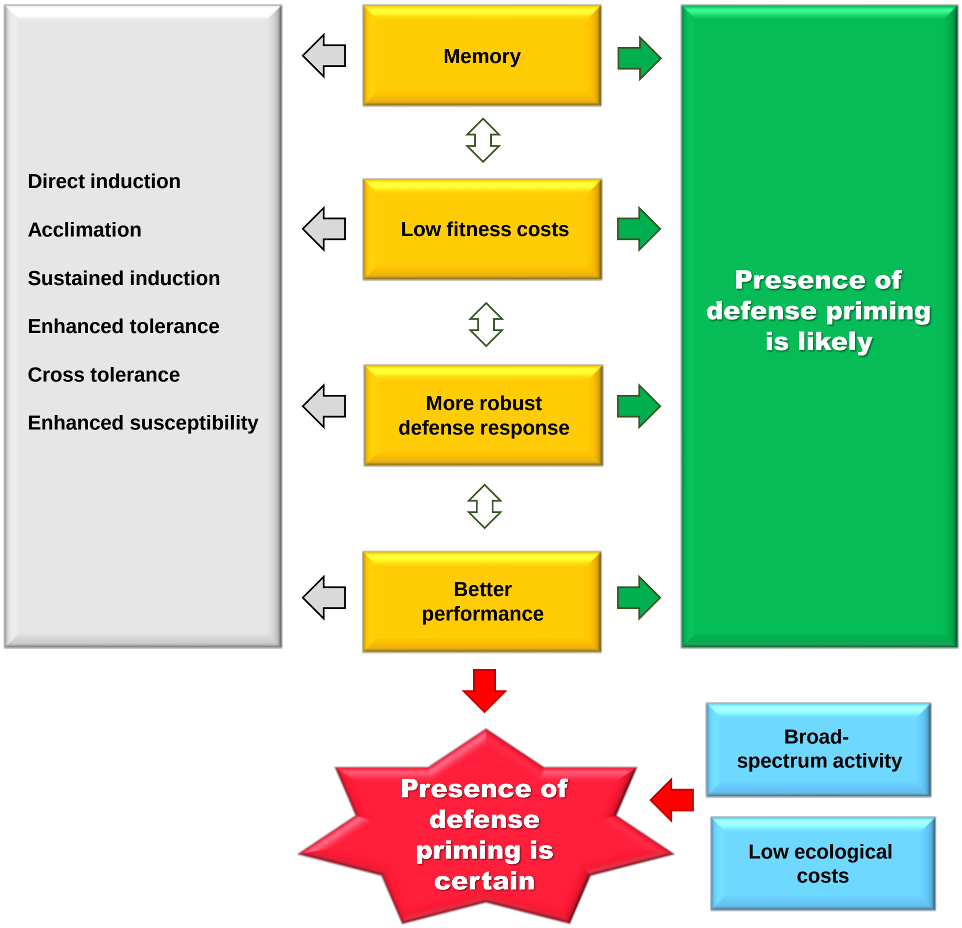

Figure 2. **Flow diagram showing the suggested guideline for analyzing presence of defense priming**. The main criteria proposed for analyzing presence of defense priming are in yellow squares. The accomplishment of such criteria (green arrows) suggest the presence of defense priming. The more criteria are accomplished, the more likely is the presence of defense priming. The grey square shows other mechanisms by which plants cope with environmental stress. They might have similarities with defense priming. These mechanisms are not exclusive, and they can co-occur with defense priming. Bright blue squares show other features of priming. Presence of these characteristics suggests presence of defense priming. However, the analysis required may represent a difficult task, especially for non-model and slow-growing plants, and would involve experiments in natural habitats.