TRACE document

Delayed chemical defense: timely expulsion of herbivores reduces competition with neighboring plants (The American Naturalist)

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TRACE document

of the TIMELY model

This is a TRACE document ("TRAnsparent and Comprehensive model Evaludation") which provides supporting evidence that our model presented in:

> **Delayed chemical defense: timely expulsion of herbivores reduces competition with neighboring plants**

was thoughtfully designed, correctly implemented, thoroughly tested, well understood, and appropriately used for its intended purpose.

The rationale of this document follows:

> Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010. Ecological modelling supporting environmental decision making: a strategy for the future. *Trends in Ecology and Evolution* 25: 479-486.

and uses the updated standard terminology and document structure in:

> Grimm V, Augusiak J, Focks A, Frank B, Gabsi F, Johnston ASA, Liu C, Martin BT, Meli M, Radchuk V, Thorbek P, Railsback SF. 2014. Towards better modelling and decision support: documenting model development, testing, and analysis using TRACE. *Ecological Modelling* 280:129-139.

and

> Augusiak J, Van den Brink PJ, Grimm V. 2014. Merging validation and evaluation of ecological models to 'evaludation': a review of terminology and a practical approach. *Ecological Modelling* 280:117-128.

0.1 Problem formulation

This TRACE element provides supporting information on: The decision-making context in which the model will be used; the types of model clients or stakeholders addressed; a precise specification of the question(s) that should be answered with the model, including a specification of necessary model outputs; and a statement of the domain of applicability of the model, including the extent of acceptable extrapolations.

Summary:

> **Time-delays in the activation of defense are thought to be the principal disadvantage of induced defenses. This suggests that all induced responses should have evolved to be very fast. However, observed time-delays between damage and defense induction in plants vary substantially. We postulate that other natural conditions, such as resource competition with conspecifics and the type of herbivores present may be important co-determinants of the cost-benefit balance for induced responses. The purpose of this model is to check whether there exists an optimal delay time τ within a population of annual plants and if this is different from zero.**

When plants are attacked by herbivores, they need to defend to prevent losing too much biomass. Plant defenses comprise toxins (like e.g. nicotine) as repellents and protease inhibitors (PIs) which reduce the growth of their insect herbivores. In addition, volatile organic compounds (VOCs) are produced which attract the herbivores' predators and hence increase the herbivore's mortality rate. However, producing defenses is costly because resources are required which could otherwise be used for growth or reproduction. Inducible defenses allow plants to forgo the costs of defenses when not needed, however, when attacked it requires a certain amount of time ("delay-time") to react and then build up the required defense compounds to its maximal value. Within this delay-time, the plant remains vulnerable. This is why time-delays in the activation of defense are thought to be the principal disadvantage of induced defenses. This suggests that there is a strong selection pressure for faster induction. However, observed time-delays between damage and defense induction in plants vary substantially, even within the same population. We postulate that other natural conditions, such as resource competition with conspecifics and the type of herbivores present may be important co-determinants of the cost-benefit balance for induced responses.

Therefore we designed an individual based model to analyze optimal delay times in a population of 400 conspecific plants attacked by different densities of mobile herbivores. The model is based on data collected from *Nicotiana attenuata* plants and its specialized herbivore, *Manduca sexta*, a lepidopteran larva. Naturally, these plants grow in dense populations and are thus in severe competition for resources. We therefore included into the model competition between plants with a two-layer ZOI approach. Plant growth follows metabolic scaling theory. The model accounts for costs of defense production and herbivory as a reduction of the maximal plant growth rate and biomass, respectively.

0.2 Model description

This TRACE element provides supporting information on: The model. Provide a detailed written model description. For individual/agent-based and other simulation models, the ODD protocol is recommended as standard format. For complex submodels it should include concise explanations of the underlying rationale. Model users should learn what the model is, how it works, and what guided its design.

Summary:

> **Please consider the ODD of the TIMELY model (see section "ODD" of the Supplement) for a complete model description.**

0.3 Data evaluation

This TRACE element provides supporting information on: The quality and sources of numerical and qualitative data used to parameterize the model, both directly and inversely via calibration, and of the observed patterns that were used to design the overall model structure. This critical evaluation will allow model users to assess the scope and the uncertainty of the data and knowledge on which the model is based.

Summary:

> **In this section the data from field work and laboratory experiments which influenced our modeling decisions is briefly presented.**

- **We measured the performance of *Manduca sexta* caterpillars on well-defended vs not-defended plants during the field season in 2014.**
- **We estimated larval mortality rates (depending on the host plant quality) in the field.**
- **We used field data of the plant's shoot masses and the number of its flowers produced to estimate whether larger plants produce more seeds (for the genetic algorithm in which we simulated several generations of plants)**
- **We got an estimation of the plant growth cycle (data provided from the greenhouse gardeners of the MPICE in Jena).**

Description of the field study

To calibrate the model, we needed information about the growth of *M. sexta* when feeding on a defenseless plant or on a well-defended plant (defined in the model as a plant which has accumulated a maximum of defense compounds). This helps estimating the impact of plant defense production on caterpillar performance. We therefore recorded the growth trajectories of 30 larvae of *M. sexta* per treatment in their native environment in the Great Basin Desert. We conducted this experiment from Mai 18th to June 10th 2014 at the Max Planck Field Station in Utah, United States of America.

We recorded larval growth on two different plant lines: either well defended, "wild type"(short "+ def") plants, or plants of the jasmonate-deficient inverted repeat allene-oxide cyclase (irAOC) line (short "- def" for "defenseless"). Jasmonic acid accumulation and perception are critical for the activation of most defense responses and the irAOC line we used in the field study shows a reduction of herbivory-induced jasmonate levels of more than 95%.

Four plants of an identical line were planted in a square-design (Fig. S1 of the supplement) in the field station of the Lytle Ranch Preserve (St. George, UT, USA). We used 30 freshly hatched 1st instar *M. sexta* larvae per plant line. Six larvae were placed per plant quadruplet. Each quadruplet was surrounded by a fluon-sprayed plastic collar of 40 cm height to protect the caterpillars against large predators (especially lizards which often feed on large larvae of the 4th or 5th instar); the collar also prevented *M. sexta* larvae from escaping. During the first days, each caterpillar was protected by a clip cage against arthropod predators; this was removed later on to allow free movement of the larvae within the quadruplet. Size [in mm] and current instar of each larva were recorded every second day, body mass of each larvae was measured, starting from the end of the second instar, on days 9, 12, 14, 17, and 19 after placement. Results were analyzed by repeated measures ANOVA to test the effect of the host plant type and fitted with a linear model of the log-transformed growth trajectory data.

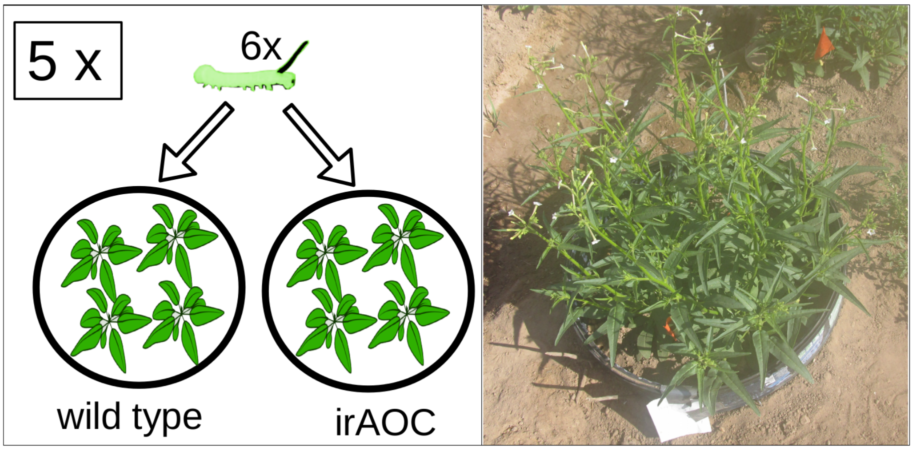


Figure ST1: Field experiment setup: The weight trajectories of *M. sexta* caterpillars on well defended (wild type) and low defended (irAOC) plants were measured. 30 freshly hatched 1st instar larvae were placed per plant line, 6 larvae per plant quadruplet. In one plant quadruples four plants of the same type (well defended or low defended) were planted. Each quadruplet was protected by a fluon-sprayed plastic ring of 40 cm height (right picture). The ring should prevent both, caterpillar escape and arthropod (lizard) feeding on caterpillars. Caterpillars were protected by a clip-cage for the first five days and could afterwards move between the plants of one quadruplet freely. The instar and weights of the caterpillars were recorded regularly.

Test equipment used

- 40 plastic rings, 40 cm height
- Fluon
- plants: 80 WT and 80 LOX3/COI1/AOC plants.
- 40 replicates for each treatment → 80 *M. sexta* eggs

Handling procedure

We placed two freshly hatched *M. sexta larvae* on one N. attenuata plant → 30 - 40 *M. sexta* larvae per plant line (LOX3/AOC/COI1 and WT). For the first 6 days, the larvae have been protected by clip cages. Starting at the 8th day after *M. sexta* placing, the weight of each larvae was measured and the length in mm was taken every second day and the current larval instar was noted.

The Manduca sexta mass performance was analyzed with repeated measures ANOVA in *gnu R* using the *aovstats* function.

Results Field Work

	not defended plant	**well defended plant**
1st instar	1 mg [1-5 d]	1 mg [1-6 d]
2nd instar	50 mg [6-8d]	40 mg [7-10d]
3rd instar	150 mg [9-11d]	130 mg [11-15d]
4th instar	1100 mg [12-16d]	900 mg [16-20d]
5th instar	2500 mg [17-21d]	2000 mg [>21d]

Table ST1: Field result: the masses and the ages (in [days]) at certain instars of larvae being fed on well defended or defenseless plants.

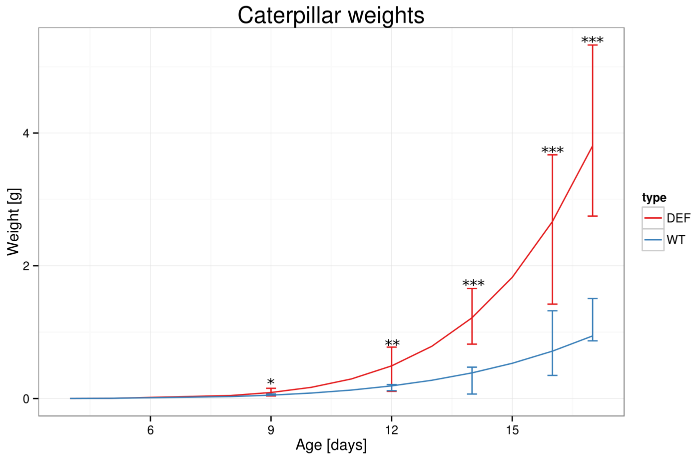


Figure ST2: The masses of *M. sexta* larvae have been measured during the field season 2014 and plotted against the larval age (in days). Larvae raised on plants being unable to produce defense compounds (red error bars, start: N = 30, end N = 16) have been compared to larvae raised on plants being maximally defended (blue error bars,start: N = 30, end N = 6). The *Manduca sexta* performances were analyzed with repeated measures ANOVA in gnu R. The lines show the fit we found by using a linear model of the log-log transformed data. The included photo shows the differences in size of a larva raised on defenseless plants (bottom) and a larva raised on well-defended plants (top).

Assessment of caterpillar survival

We assessed caterpillar survival in the field during the larva performance experiment. As we used clip cages to protect the larvae during the first six days of the measurements (which reduces mortality rates) we estimated a lower number of surviving caterpillars than recorded for the first six days.

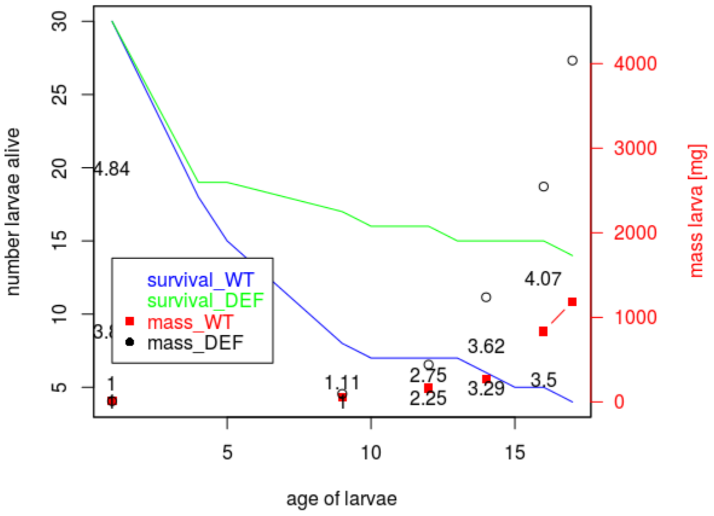


Figure ST3: Estimated survival chances of *M. sexta* larvae in the field. In green: number of surviving larvae feeding on not-defended plants, in blue: number of surviving larvae feeding on well-defended plants (both numbers starting with 30 larvae). In red and black: the mean of masses of the caterpillars (red: on not-defended plants, black: on well-defended plants). It shows that mass scales anti-proportional to mortality.

Growth cycle of Nicotiana attenuata in the greenhouse

Figure ST4 shows how many days it takes for a plant to grow to certain developmental stages. This figure is based on observations of the greenhouse gardeners. As our model is only dealing with plant-caterpillar interactions, it does not consider the germination and rosette stage and starts when plants are beginning to produce flowers and weigh about 30 g (fresh mass above ground).

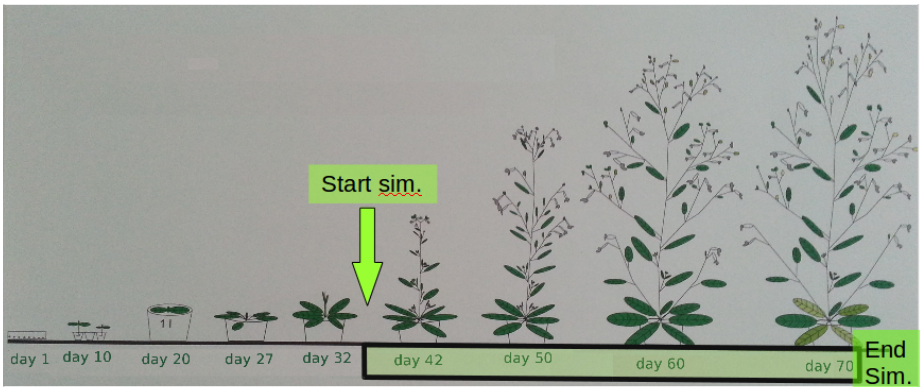


Figure ST4: The estimated growth cycle of Nicotiana attenuata. The time when the simulation starts (when the plants start to flower) and when it ends (when plant senescence begins) are marked on the x-axis.

This corresponds to the time where in nature *Manduca sexta* moths are attracted to the floral scent of the first flowering plants and start with oviposition (this happens approximately at the end of month of May in southern Utah). Oviposition is a process which mostly happens in waves. The first wave takes place at the end of May and the second wave some weeks later when the newly hatched moths start laying eggs. One wave of oviposition happens in consecutive nights, however in order to simplify this process all moths lay their eggs at the same time on the plants in the model (but see our Robustness Analysis in the supplementary file, where we relaxed this simplifying assumption). The simulation ends 27 days afterwards, at a time when plants start to wilt and larvae have left the plants for pupation. At this time the plants have reached their final size, the largest ones can weigh between 300 and 400 g (above-ground fresh mass), more common is about 150 - 250 g.

Relationship of plant mass and fitness

Fig. ST5 shows that there is a (more or less) linear relationship between plant mass and plant fitness (as number of flowers produced). Therefore we use the plant's productivity (sum of above-ground masses at the end of the simulation) as a proxy for plant fitness. Fig. ST5 shows as well the typical above-ground mass distribution reached by plants in the field at the end of a season. This mass distribution is used in the model's analysis as a reference value.

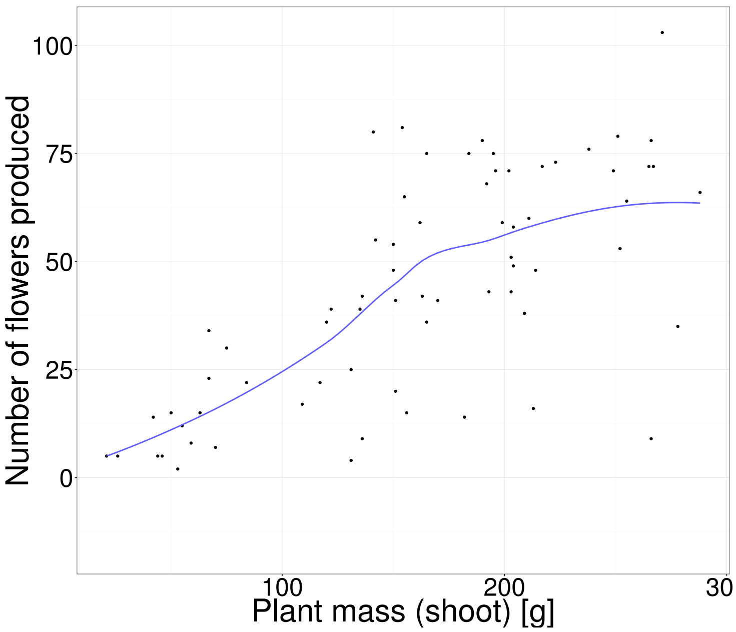


Figure ST5: The plant's mass roughly scales linearly with the number of flowers produced at the end of the season. Source: 69 field samples taken by Arne Weinhold at the end of the field season 2013. It shows as well that large plants reach above-ground masses of up to 300 g

0.4 Conceptual model evaluation

This TRACE element provides supporting information on: The simplifying assumptions underlying a model's design, both with regard to empirical knowledge and general, basic principles. This critical evaluation allows model users to understand that model design was not ad hoc but based on carefully scrutinized considerations.

Summary:

> **Here we discuss the simplifying assumptions underlying our conceptual model. Most uncertainties of the model concerned the behaviour of caterpillars. Here we only got estimations from field observations but no exact numbers. Therefore we tested for different options whether or not the modeling results mirrored the patterns observed in nature.**

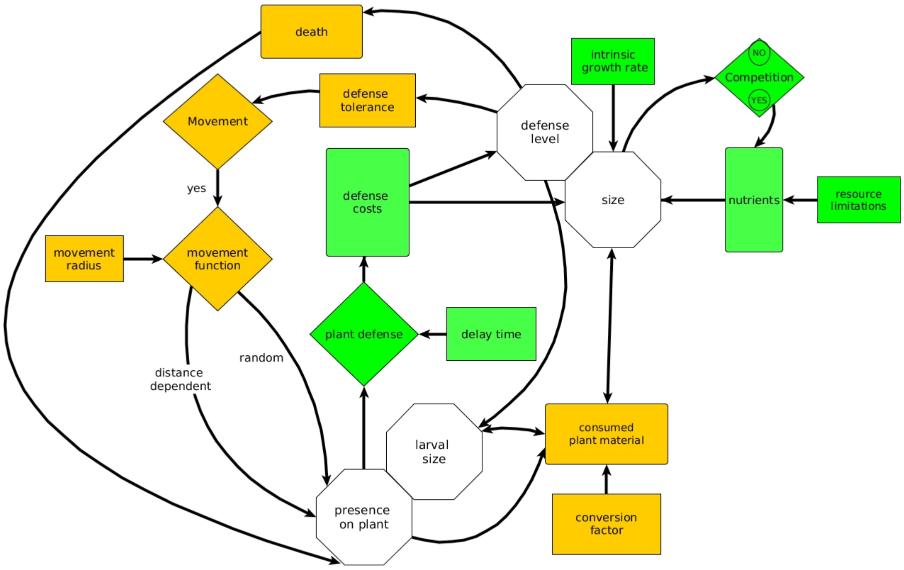


Figure ST6: Conceptual representation of the TIMELY model. Orange fields represent caterpillar-related variables, constants or procedures, green fields are plant related. Rectangles show constants which are set before the simulation, rectangles with rounded corners show variables which are calculated within the simulation and diamond-shaped fields show procedures or mechanisms. Octagons represent the state variables of either plant (top-right) or larva (bottom).

How far can larvae move in-between plants (= movement-radius)?

When the larvae have reached a certain size and instar, they are able to switch their host plants. They do this either, when the former host plant is entirely consumed ([Casey, 1977]), or when the defense level of the host plant is too high ([Van Dam et al., 2000]). In the field I observed that most larvae go to the nearest plants if they switch plants. However, 5th instar larvae can move up to several 100s of meters when it is required ([Kessler and Baldwin, 2002]). The latter seems to happen when larvae are searching for a place to go into pupation, at this time they have the urge to move as far as possible away from their former host plant. In normal cases, however, every inter-plant movement exposes the larva to predation. Therefore larvae prefer neighbouring plants as potential host-plants, despite of being physiologically capable to cover larger distances. Therefore we chose for larvae a maximum movement diameter of 4 m (the furthest distance the author noticed for non-pupation larvae). As the modelling world is 250 x 250 pixels big which corresponds to an area of about 15 x 15 meters, this means a movement radius of 4 m corresponds to about 70 pixels of the modeling world. In average, about 90 plants are within a movement radius of 4 m, thus nearly 1/4 of the whole plant population.

Remark: I chose periodical world boundaries in the model to avoid edge effects. Only for the single plots of movement trajectories the world has been set to “fixed boundaries” for easier plotting purposes.

How do larvae choose the next plant in the model?

From the larva’s viewpoint, two criteria are relevant for chosing the next plant:

1. chose a plant with a high ”quality” thus a defense level which is as low as possible
2. minimize the distance between current and future host plant

1. Host plant quality

It has never been shown that larvae are capable of recognizing the quality of a host plant before feeding on it. During two field seasons of 8 weeks I did not get any clue that larvae *do* chose plants according to their quality. To me it rather seemed as an uncoordinated movement to the next plant which is roughly in movement direction (however, larvae do not forcibly chose the closest neighbour plant, though). This means that there certainly is a stochastic element included - the choice of the next host plant depends on the direction the larva is facing when having descended the former host plant.

Because of this I decided **not** to include a larval preference for plants with a low defense-level.

2. Distance between plants

Movement between plants increases starvation and predation risks (Schultz 1983, Bernays 1997) and therefore movement distance should be reduced as much as possible. Therefore we included distant-dependent movement function in the model, in which the possibility for the larva to chose a plant (among all plants within their movement radius) is inversely proportional to its distance of the larva's current position (see Fig. ST7). Please note that movement is not explicitly represented in the model, but only its outcome, i.e. the switch from one plant to another.

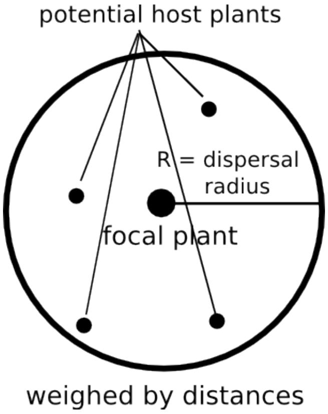
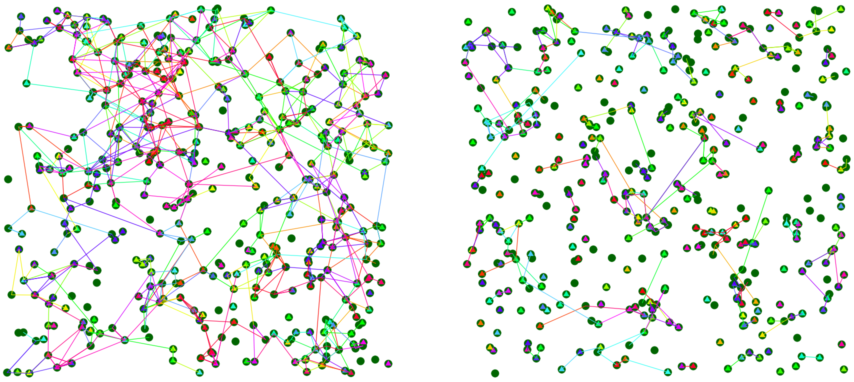


Figure ST7: The movement radius of a larva determines to which plants it can potentially move. The next plant within that area is chosen by chance with a probability which is inversely correlated to the plant's distance to the current larval position.

I tested several distance-dependent plant choice functions:

1. the probability of choosing the next plant decreases **linearly** with distance.
2. the probability of choosing the next plant decreases **cubically** with distance.
3. the probability of choosing the next plant decreases **exponentially** with distance.

For each option, the simulated movement trajectories were plotted and compared the to observations made in the field.



(a) (b)

Figure ST8: Movement trajectories of larvae during one simulation of 30 days, with 300 larvae and 400 plants. Plants are symbolized by dark green circles. Each line with a different color represents a different larva. Larvae are at the beginning of the simulations distributed randomly on the plants (the initial position of each larva is shown with a triangle), all further movement is shown by a line. When a line is present it means that a larva moved at least once between both plants, repeated movement can be possible, too. (a) movement function depends linearly on distance (b) movement function depends cubically on distance

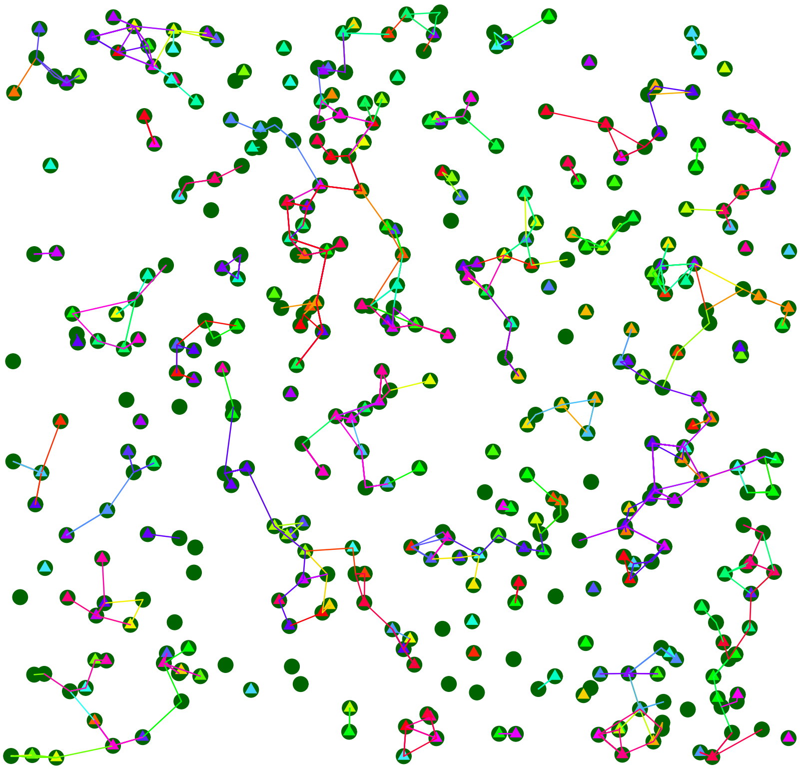


Figure ST9: Movement trajectories of larvae during one simulation of 30 days, with 300 larvae and 400 plants. Here, larvae chose their next host plant with a probability which decreases exponentially with the plants' distance from the larva. Plants are symbolized by dark green circles. Each line with a different color represents a different larva. Larvae are at the beginning of the simulations distributed randomly on the plants (the initial position of each larva is shown with a triangle), all further movement is shown by a line. When a line is present it means that a larva moved at least once between both plants, repeated movement can be possible, too.

In figures ST8, larvae moved between several plants which were up to four meters apart from each other. Sometimes the larvae ignored nearer plants on their path which does not seem realistic: We observed in the field that a larva might ignore a nearby plant when it is facing a significantly different walking direction, however it would not directly pass a plant (as it happened in the simulation).

One can see that in figure ST9 most larvae tend to stay in a very restricted area of about 2-4 plants. Sometimes larvae do not switch plants at all. In the plot all larvae are shown, even those which died after some days of simulation before reaching the mobile stage in their development. (About 2/3 of all larvae die during the simulation).

The exponential movement function mirrors best the conditions observed in nature. Therefore the movement function included considers all plants within the movement-radius of the larva and choses among them a plant by chance. Here, the probability P_i of plant i to be chosen decreases exponentially with its distance to the larva's current position:

$$P_i = \frac{exp(\text{distance}(i))}{\sum_i exp(\text{distance}(i))} \tag{1}$$

Mortality
If a larva stays on a plant, the mortality depends on its size and the quality (thus defense-level) of the plant. The smaller the larva, the higher the probability of being predated (because the predators *geocoris spp.*of the larvae are small, too). The higher the defense-level of the host plant, the more toxins have to be digested by the larva which results in slower growth and more sluggish and slow movement, thus a decreased ability to defend itself against predator attack. As well, the induced plant emits volatiles to attract predators and thus raises the probability that a larva is detected by a predator.

A larva which is on plant (P_i) with defense-level $Def(P_i)$ has the following death probability deathprob(t) for the next time step (1/6 day):

$$\text{mortality}_l(t) = \frac{(\text{death coefficient} + 1.5 \cdot \text{Def}(P_i) - 0.1)/6}{1 + log(\text{biomass}_\text{larva}) \cdot exp(1)} \tag{2}$$

The default death coefficient is set to 0.25. If the larva moves between plants it receives – as switching plants is more dangerous than staying put – a mortality penalty which depends on the distance the larva travels. The further the larva moves, the higher the mortality:

$$\text{mortality}_l(t) = \frac{\text{death coefficient} + (\text{distance} * 1.5/\text{movement}_\text{radius})/6}{1 + log(\text{biomass}_\text{larva}) \cdot exp(1)} \tag{3}$$

Moving off a defended plant vs staying
If a larva moves between plants, it has (during one time-step) a higher death probability than when it stays put. Under which circumstances is movement of advantage?
One option is that the plant in entirely consumed or wilted. In that case moving is indispensable for survival. However, we observed in the field that larvae also leave their host plants when there is still plant biomass left. Thus we conclude that it benefits the larvae under certain circumstances to leave a plant: Imagine the larva is on plant (P_i) which has the maximum defense level the larva can endure ($Def = 0.24$). This leads to a slower larval growth rate and a high mortality chance. Moving to another plant would mean that the larva encounters on average a new host plant with a lower defense level, resulting in a higher larval growth rate and lower mortality. However, moving means a distance-dependent higher mortality rate for the time the larva commutes.
Would this pay off for the larva?

To calculate this, we need the mean distance a larva covers when switching plants (because the death-penalty for moving is distance-dependent) and the mean defense-level of all plants (because the death-probability scales with the defense level of the host plant). In Fig. ST10 the mean distance per inter-plant movement of one larva is shown (100 simulation runs).

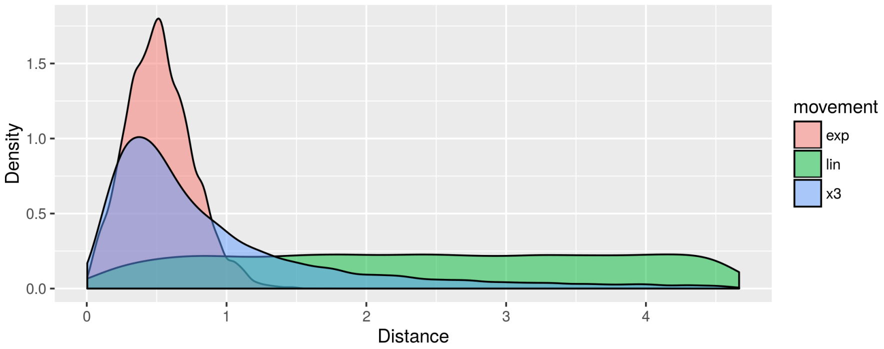


Figure ST10: Density distribution of the distances covered per larva when switching plants (in m). In green: the probability to chose a plant as next host plant decreases linearly with distance, blue: it decreases cubically with distance and red: it decreases exponentially with distance.

The mean defense level of all plants (over 100 simulations) depends as well on the movement strategy of larvae: the further larvae move, the higher the mean defense-level of plants (Fig. ST11). This is interesting as there are less surviving larvae when moving further, so one should expect a higher overall defense level of plants. This indicates that the plant's strategy of sending the larvae away is more efficient when the larvae attack nearby plants. "More efficient" means in this context that the plants need to produce less defense compounds for a similar effect on the larvae.

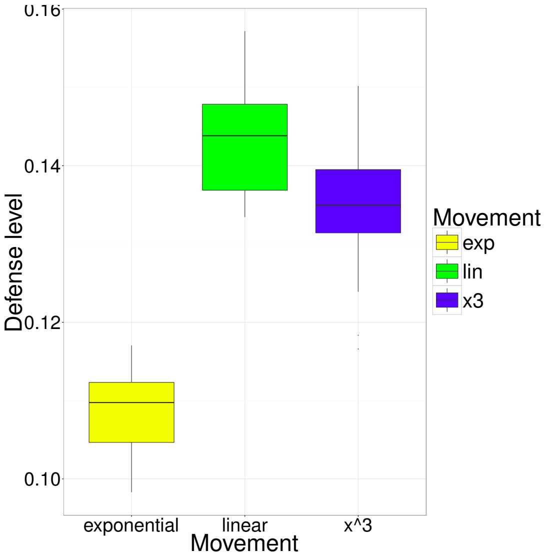


Figure ST11: Comparison of the mean defense-level of all plants for simulations with different movement patterns of larvae.

Movement function	Mean distance	Mean defense level
linear	2.42 m	0.144
cubical	0.94 m	0.135
exponential	0.52 m	0.109

Figure ST12: Results of the 100 simulation runs of the different movement functions. The mean distance between host plants is given (middle) and the mean defense level of all plants at the end of the simulation (right column).

Now we can compare the mortality chance (for 1 simulated day) for a larva which moves with the mortality of a larva which stay on a highly defended plant:

1. Larva stays on highly defended plant:

$$\underbrace{6/6 \cdot (0.25 + 1.5 \cdot 0.24 - 0.1)}_{\text{penalty for plant defense}} \Rightarrow 51\% \tag{4}$$

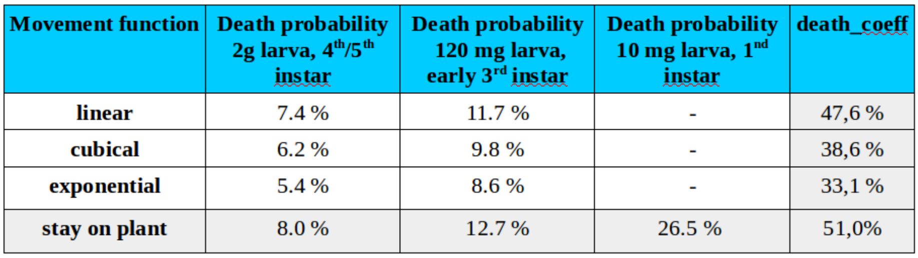
Linearly-dependent choice of plants:

$$\underbrace{1/6 \cdot (1.5 \cdot 2.42m/4.67m + 0.25)}_{\text{penalty for movement}} + \underbrace{5/6 \cdot (0.15 + 1.5 \cdot 0.144)}_{\text{penalty for plant defense}} \Rightarrow 0.171{+}0.305 = 47.6\% \tag{5}$$

Exponentially-dependend choice of plants:

$$\underbrace{1/6 \cdot (1.5 \cdot 0.52m/4.67m + 0.25)}_{\text{penalty for movement}} + \underbrace{5/6 \cdot (0.15 + 1.5 \cdot 0.109}_{\text{penalty for plant defense}} \Rightarrow 0.695{+}0.261 = 33.07\% \tag{6}$$

If the larva stays on the plant for one day, it has a probability of 51 % to die, if it moves away, it has in the worst case (when it moves far)a 47.6 % mortality chance.



Movement function	Death probability 2g larva, 4th/5th instar	Death probability 120 mg larva, early 3rd instar	Death probability 10 mg larva, 1nd instar	death_coeff
linear	7.4 %	11.7 %	-	47,6 %
cubical	6.2 %	9.8 %	-	38,6 %
exponential	5.4 %	8.6 %	-	33,1 %
stay on plant	8.0 %	12.7 %	26.5 %	51,0%

Figure ST13: Being on a well defended plant: The death probabilities for a day for different options: either the larva stays on the plant for one day (stay on plant), or it moves either with a according to a movement function in which the next host plant is chosen by a probability which scales linearly, cubically or exponentially anti-proportional to distance. In the time the larva moves between plants, it is sentenced in all cases with a death-penalty which scales with distance. As mortality depends on the mass of the larva, different scenarios (small, mobile larvae (3rd instar), big, mobile larvae (5th instar) and very small, non-mobile larvae (2nd instar) are considered. In the latter case, no death probabilities for movement options have been calculated, because larvae start moving when being in the third instar.

Conclusion

Larvae tend to stay on a plant for a long time, even when the host plant becomes more and more unpalatable. When however a certain defense-level is reached in the plant, the probability of dying (over time) becomes greater than the death penalty for moving.

Consequences of plant switching for larvae

Larvae switch their host plants for two reasons: either the host plant has been eaten (meaning only 10 g on plant mass remains because larvae usually do not feed on stalks) or the host plants defense level exceedes the threshold of the larva.

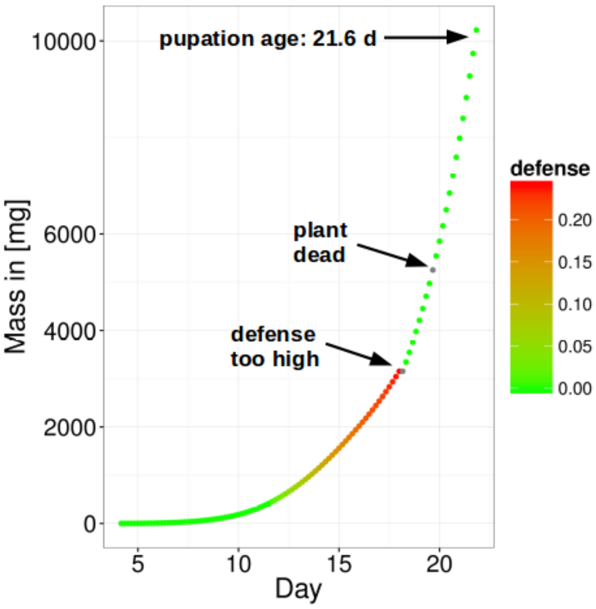
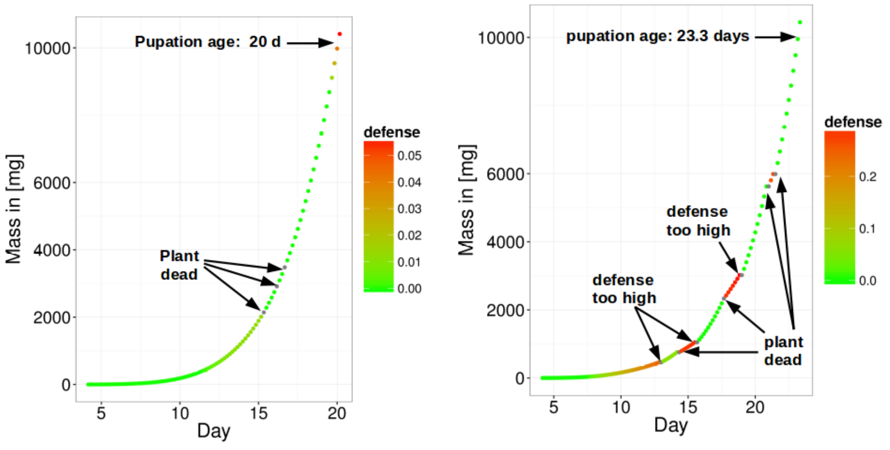


Figure ST14: The weight of a single larva during one simulation plotted over time. The colors of the points show the defense-level of the larva's current host plant. Red means, that the host plant has produced a large amount of defense compounds, green means that the plant is relatively defenseless. Grey dots show that a larva currently is switching from one plant to another. The reason for switching (either the host plant defense level has raised too high [defense-level > 0.24] or the host plant has been eaten) are indicated in the figure. As well the pupation age of the larva is recorded. Please note that about 2/3 of all larvae die before pupation.

As we have seen in the former section, moving away from a host plant when its defense-level is very high increases the survival probability of the larva. It as well increases the speed of growth of the larva because digestion is inhibited by defense-compounds. Fig. ST15 (b) shows this effect: the curve of mass gain is much shallower if the host plant is well defended. As growth on induced plants is delayed, as well pupation'stage is reached later (see Figs. ST14 and ST15).



(a) (b)

Figure ST15: The weight of a single larva during one simulation plotted over time. The colors of the points show the defense-level of the larva's current host plant. Red means, that the host plant has produced a large amount of defense compounds [defense-level > 0.20], green means that the plant is relatively defenseless. Grey dots show when a larva is switching from one plant to another. The reason for switching (either the host plant defense level has raised too high [defense-level > 0.24] or the host plant has been eaten) are indicated in the figure. As well the pupation of the larva age is noted. (a): a larva which mostly encounters host plants with low defense-levels; (b) a larva which encounters both well and low defended host plants.

During field observations I recognized that if larvae have a choice when to switch plants they prefer moving during nighttime to avoid predation and as well to move when the sand was cooler (temperatures are quite high during the larval growth season in Utah). Therefore, when the maximum defense level of the host plant is reached, the larva only switches its host plants when it has stayed for at least a full day on the plant.

Switching statistics

We recorded the mean number of plant-switches per larva, the mean number of different plants visited by a larva (this should be less or equal the first, as a larva can visit a plant several times if convenient) and the percentage of the number of switches which were motivated by a high defense level of the plant (see Tab. ST2).

mean defense	# switches	defense-switches	# unique	pupation age	mortality
0.086	5.33	30.0 %	4.1	115.8	69.8%
0.105	5.38	36.5%	3.9	121.3	73.8%
0.123	5.45	36.9 %	3.4	134.7	86.4%

Table ST2: Switching statistics for simulations with different defense-levels of host plants. **Mean defense** = mean defense level of plants; **# switches** = mean number of host plant switches of larvae during one simulation; **# defense switches** = the mean number of switches in one simulation per larvae which were motivated by a too well defended plant and not an eaten plant; **# unique** = how many different plants did the larvae encounter (mean); **pupation age** = mean number of days until the larvae pupated; **mortality** = mean larval mortality

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0.5 Implementation verification

This TRACE element provides supporting information on: (1) whether the computer code implementing the model has been thoroughly tested for programming errors, (2) whether the implemented model performs as indicated by the model description, and (3) how the software has been designed and documented to provide necessary usability tools (interfaces, automation of experiments, etc.) and to facilitate future installation, modification, and maintenance.

Summary:

> **While programming the model, the source code has been built modularly and each new procedure has been thoroughly checked by several tests, included syntax checking of the code, visual testing through NetLogo interface, the use of print statements and spot tests with agent and patch monitors.**

Testing our implemented Genetic Algorithm

Comparison with an other algorithm
We compared our genetic algorithm with a simple algorithm found in the netlogo models library “Simple Genetic Algorithm”.

The genetic algorithm used for comparison is composed of the following steps:
1) A population of plants with random τ values is created.
2) Each τ value is evaluated on the basis of how well it solves the problem, thus how much shoot biomass the plant can produce within one simulation. This measure of the “goodness” of the solution is called its“fitness”.
3) A new generation of plants is created from the old generation, where solutions that have a higher fitness are more likely to be chosen as “parent” solutions than those that have low fitness scores.
  A) The selection method used in this model is called “tournament selection”, with a tournament size of 3. This means that 3 solutions are drawn randomly from the old generation, and the one with the highest fitness is chosen to become a parent.
  B) The drawn parent creates a child and the child is a clone or copy of the parent.
  C) There is also a slight chance that mutation will occur, which means that the child is given a different τ value drawn from a uniform distribution $\in [0, 10]$ days.
4) Steps 2 and 3 above are repeated 300 times.

We assumed that in general, both GAs should lead to similar conclusions.

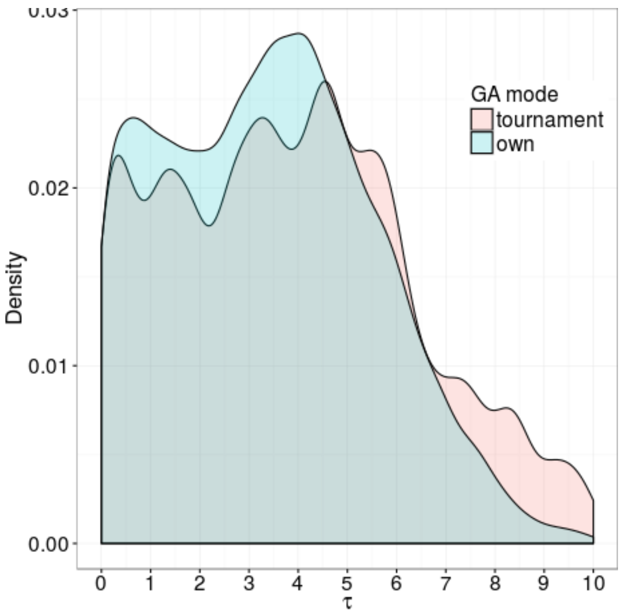
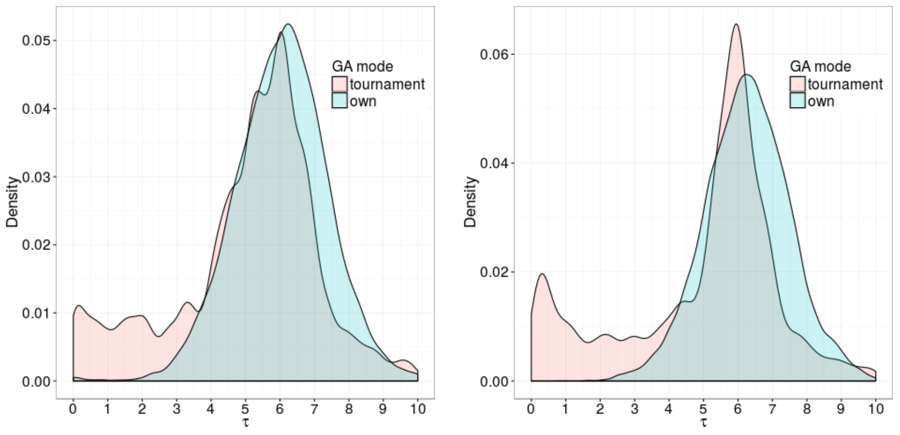


Figure ST16: Comparison of two different Genetic Algorithms: In blue: resulting frequencies of the τ-values after 300 generations when the Genetic Algorithm which was written by the author of the study is used,in red: results of a Genetic Algorithm taken from the netlogo library which uses a “Tournament selection”. Result of simulations with 200 larvae.

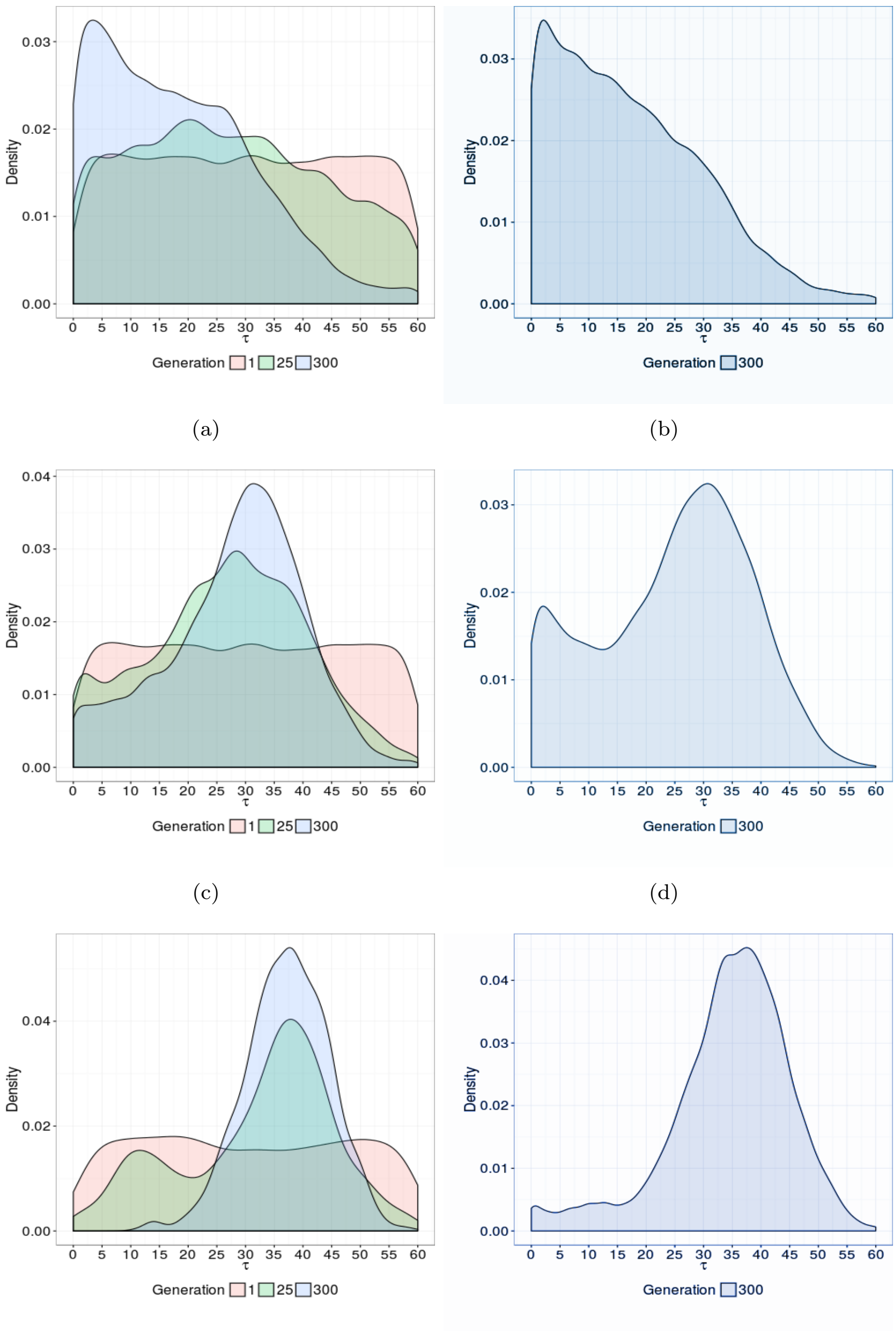


(a) (b)

Figure ST17: Comparison of two different Genetic Algorithms: In blue: resulting frequencies of the τ-values after 300 generations when using the Genetic Algorithm written by the author of the study, in red: results of a Genetic Algorithm taken from the netlogo library which uses a “Tournament selection”. Left panel: simulations with 400 larvae. Right panel: simulations with 500 larvae Both algorithms yield similar values, the algorithm written by the author results in a smoother curve of the distribution, which is a result of a lower mutation rate used.

Forward vs backward genetic algorithm

We recorded the mean delay times of the 300th generation ob the genetic algorithm (in which all plants started with random values τ drawn from a uniform distribution $\in [0, 10]$ days). As a second step we initiated all plants with the same value of τ (the result of the genetic algorithm) and let the algorithm run again for 300 generations, to see whether the same frequency distribution of delay times was reached in the last generation. After 300 generations, the genetic algorithm leveled out to the same resulting frequencies of the stationary distribution found for the GA starting with the uniform distribution of τ (Fig. ST18).



(a) (b) (c) (d) (e) (f)

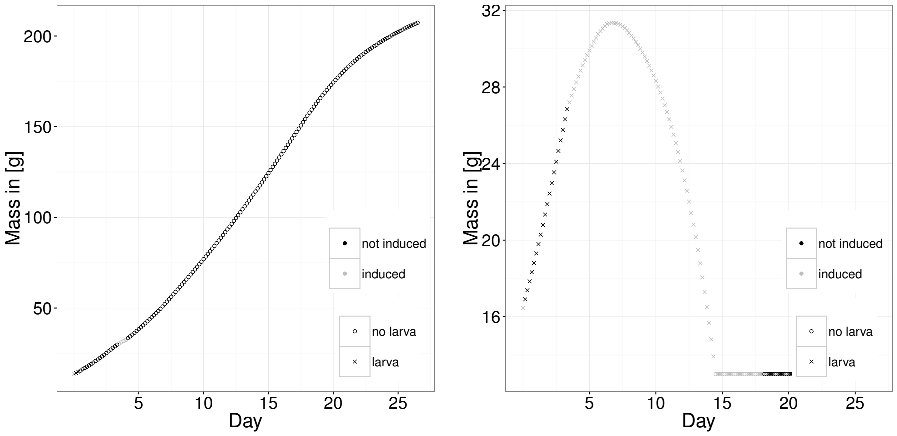
Figure ST18: Left panels: Result of the normal ("forward") genetic algorithm starting with random τ values at the beginning showing how the frequency distributions of τ values emerge with generations. Right panels: Backwards-genetic algorithm: all simulations started with the same τ value for all plants: the mean of the resulting distribution of the simulations shown in the left panel. So the beginning standard deviation was 0, after 300 generations, a frequency distribution similar to the forward GA re-emerged.

0.6 Model output verification

This TRACE element provides supporting information on: (1) how well model output matches observations and (2) how much calibration and effects of environmental drivers were involved in obtaining good fits of model output and data.

Summary:
Here we looked at the larval performances on well defended and not defended plants and compared them to our observations made in the field. We also checked the plant growth and defense parameters used in the model for realism.

Plant defense and growth



(a) (b)

Figure ST19: Growth patterns of two different plants in the same simulation. Circles mark time points where no larva was on the plant,"x" marks larval infestation. If the symbols are grey, the plant is induced and produces defense compounds. Left: On this plant, only in the first day of the simulation a larva is present. As small larvae do not feed much biomass, the plant nearly receives no damage. It can thus grow fast until competition becomes more important because it neighbouring plants reached a certain size. Right panel: This plant is attacked from the beginning on by a larva. After a certain delay time of 3 days it starts producing defense compounds(this additionally slows its growth down). Defense production does not start soon enough to repel the larva so the larva continued feeding and growing until the plant got entirely consumed (if the plant above-ground mass falls beneath 10 g, the plant is considered as dead).

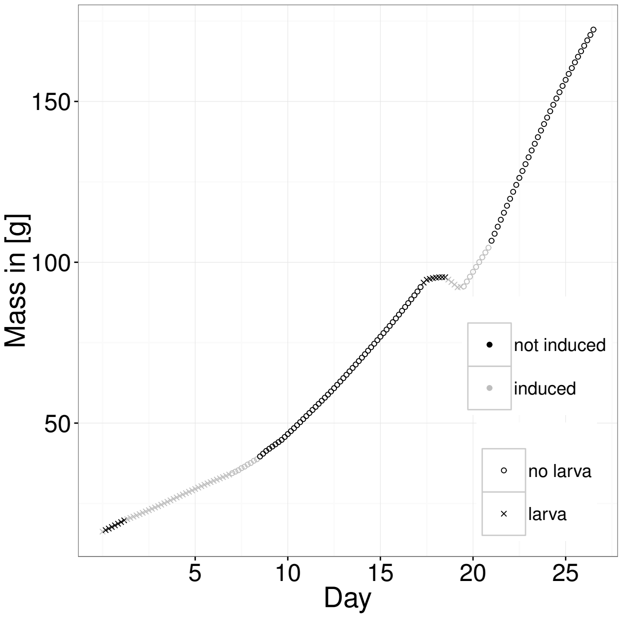


Figure ST20: Growth of a plant during the simulation. Circles mark time points where no larva was on the plant, “x” marks larval infestation. If the symbols are grey, the plant is induced and produces defense compounds. One can see that after a delay time of one day, the plants reacts to larval feeding. When the plant is induced its growth curve is shallower because 30% of available resources are put into defense production. When larvae are present, biomass is removed from the plant. The amount of mass a larva consumes per day scales with larval size, so the infested plant either grows slower (when the larva is still small) or the plant’s mass decreases when the larva has reached a certain size. In this figure, one can see that plant’s defense production eventually kills the larva or drives it off the plant.

0.7 Model analysis

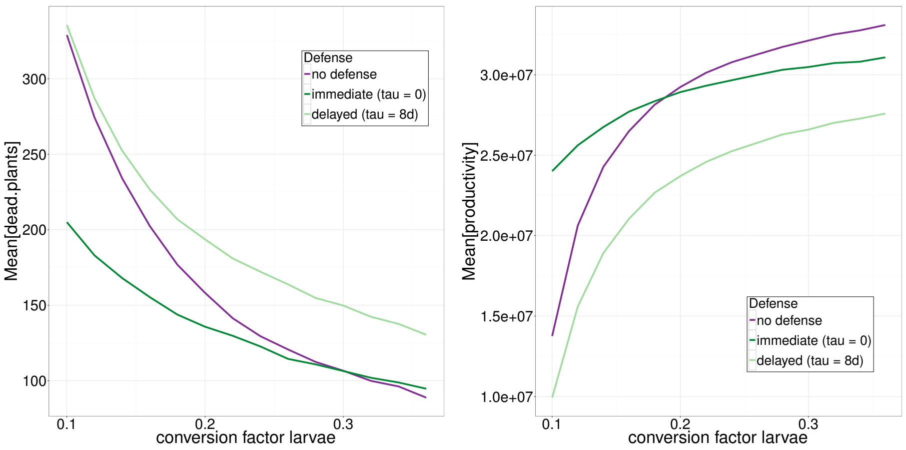
This TRACE element provides supporting information on: (1) how well model output matches observations and (2) how much calibration and effects of environmental drivers were involved in obtaining good fits of model output and data.

Summary:
We performed a Robustness Analysis to estimate the limits of our model and then we performed a Sensitivity Analysis to estimate the sensitivity of the model to different parameterizations.

Robustness Analysis

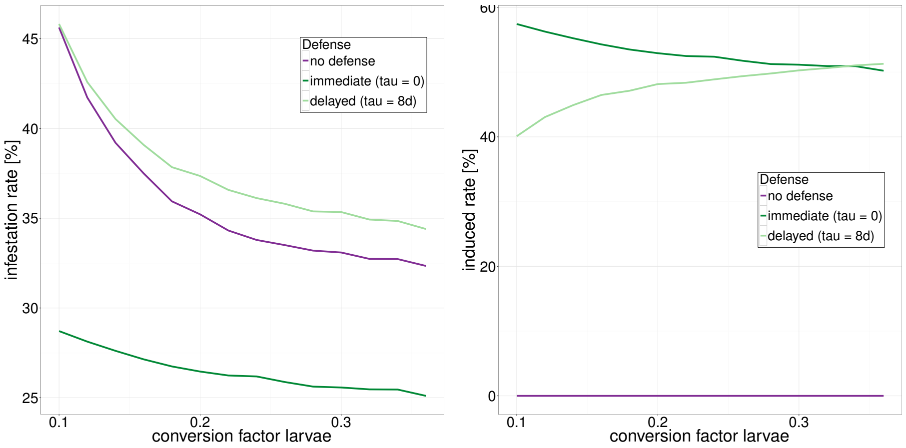
Our model explores a general question, whether delaying chemical defense of herbivores can improve plant fitness, given the plants grow in dense cohorts. However, the TIMELY model has been tailored in terms of parameter values and functional relationships to mimic the *N. attenuata* - *M. sexta* system. Therefore, we performed additional simulation experiments that test the model's behaviour in a more general context and will discuss implications for general theory. We used the rationale of "robustness analysis" ([Levin, 1966]; see above; [Grimm and Berger, 2016]): using a wider range of settings in terms of parameters and functions and also including even unrealistic values to try and "break" the model and thereby explore the robustness of the main findings and identify key components of a model system's organization. All simulations performed for the robustness analysis are listed in table S2 of the supplementary file.

Relationship of plant defense and the caterpillar's conversion factor



(a) (b)

Figure ST21: Comparison of the interactions of different imposed delay times and the conversion factor of caterpillars: light green equals a long delay time of eight days, dark green is an immediate plant reaction and violet means no defense reaction at all ($\tau \to \infty$). Left figure: The mean number of dead plants. Right: Effect on the productivity of plants. It shows that the conversion factor of larvae should not be > 0.18 if defense production should pay off at all for the plant.



(a) (b)

Figure ST22: Comparison of the interactions of different delay times of defense and the conversion factor of caterpillars: light green equals a long delay time of eight days, dark green is an immediate plant reaction and violet means no defense reaction at all ($\tau \to \infty$). Left figure: Effects on the mean infestation rate of plants. Right figure: Effect on the rate of induced plants over time.

Please remark that these simulations (Figs. ST21 - ST22) do not contain mixed variations of plants (either all plants have a long delay time or none). Even though plants with a longer delay time show a weaker performance in the figures, a long delay time can still be an advantage if combined with plants with shorter delay times (→ frequency dependent selection).

Larvae which move randomly have a higher mortality rate than those switching to neighbouring plants only (Fig. ST23). While moving on the soil between plants, caterpillars are more easily detected by their predators (most of the predators are prefer walking on the ground, like *Geocoris spp.*, several ant and spider species). If the random modus is chosen, 248 of 300 larvae die (mean of 100 simulations), a number which does not match our estimation of larval mortality in the field (about 2/3 larvae die). This movement scenario results in a higher plant productivity and less dead plants because of lower larval pressure.

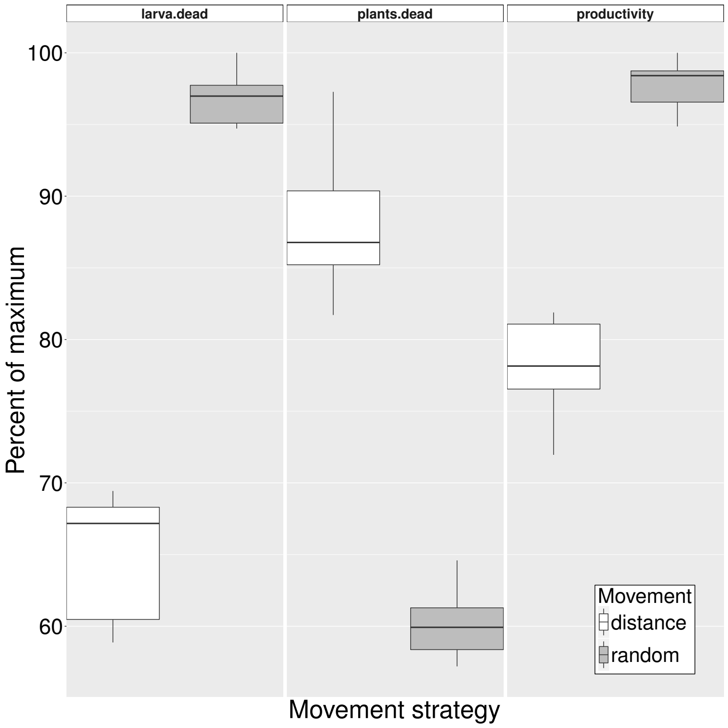


Figure ST23: Two larval movement strategies are compared. White boxes: the larva choses its next host plant with a probability which scales inversely exponential with distance, meaning that closer plants are preferred. Grey boxes: the next plant is chosen randomly out of one of the existing plants. We compared the two movement strategies for three measures: 1. The mean number of dead larvae during simulations (left column). Simulations were performed with default parameters, thus 300 larvae. 2. The mean number of plants which died during the simulations (default number of plants is 400) and 3. The productivity, thus the sum of shoot biomass of all surviving plants. The values are relative values to show the differences in % of the maximum measured.

As *M. sexta* larvae do only switch between plants when they have already reached a certain size ($\leq$ 3rd instar), we hard-coded this into our model. However, this threshold is system dependent – in the case of *M. sexta* small individuals cannot move on the hot desert sand – and therefore tend to stay put on the plants. We are aware that for many herbivore-plant systems there is no such threshold. Therefore, we also included the possibility to simulate larvae which are able to switch all the time into the model. We compared the resulting mean delay times for plants being challenged by larvae which are mobile only since the third instar and plants being challenged by larvae which are mobile all the time. We here found that the latter case resulted in even longer delay times (Fig. ST24).

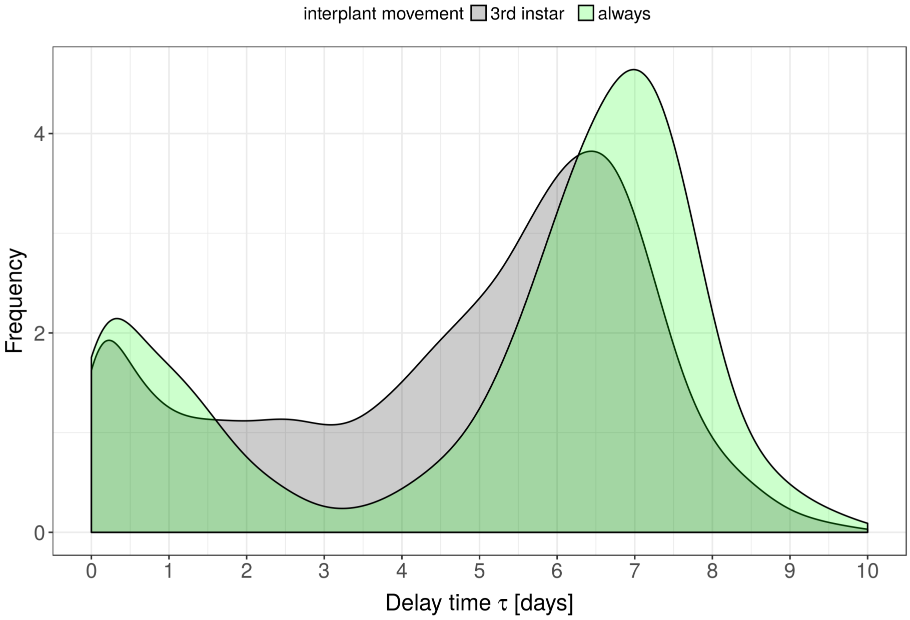


Figure ST24: Comparison of emerging delay times for plant populations challenged by A) herbivores which are mobile all the time (green) and B) herbivores which are mobile only after reaching a certain weight/instar (grey).

The robustness analysis shows that the combination of the following points are crucial for using a “delay strategy”:

- mobility of herbivores[1]
- competition of plants (inter- or intra-specific)
- shared herbivores
- herbivores attack the plants in waves[2]

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[1] We observed two mobility types: 1) Immediately: the larva can switch plants at any time. 2) At a certain age: The larva can leave the host plant when it reached the 3rd instar.

[2] ”waves” mean in that context that larvae are oviposited at the same time, however these oviposition events happen several times throughout one simulation.

We therefore are convinced that our main conclusion holds: the generally held assumption that damage done by herbivores before the defense production is induced are always costly cannot be sustained. In that sense, the model addresses a general hypothesis that now is refuted.

Sensitivity Analysis

Sensitivity analysis are several standardized methods used for the calibration of the model's parameters. It estimates the sensitivity of the model's outcomes to parameter changes. Like this one learns about the robustness of the model due to parameter uncertainty. Furthermore it helps understanding the relative importance of mechanisms in the model. ([Saltelli et al., 2008] and [Thiele et al., 2014]).

The following methods were applied:

1. Full Factorial Design
2. Global Sensitivity Analysis

All analyses were performed by combining gnu R (using the packages RNetLogo, DoE, FrF2, MASS) and netlogo (see [Thiele, 2014] for more information).

Global Sensitivity Analysis: Design of Experiment

A full factorial design of the two extreme values of each of the parameters being tested was chosen. The input parameters are shown in table ST3.

input parameter	**min. value**	**max. value**	**default**
intrinsic growth rate (plants)	0.2	1.3	0.8
defense fraction (plants)	0.1	0.5	0.3
conversion factor (larvae)	0.1	0.5	0.18
dispersal radius (larvae)	0.07 m	6.7 m	4.7 m
death-coefficient (larvae)	0.1	0.5	0.25
τ-median (plants)	0	80	30
τ-range (plants)	0	60	20

Table ST3: Parameters used by the global sensitivity analysis. These parameters were given extreme values to test the model's sensitivity for them.

The input parameters were tested for their effect on certain output values of the model. The output values are shown in table ST4.

productivity	**dead.larvae**	**dead.plants**	**infestation rate plants**
$\sum_{\text{time}} (B_{\text{above}})$	$\sum_{\text{time}} (\text{dead larvae})$	$\sum_{\text{time}} (\text{dead plants})$	$\sum_{\text{time}} \left(\sum_{\text{plants}} \left[\frac{\text{plants with larva}}{\text{plants alive}} \right] \right)$

Table ST4: Output values which are used in the global sensitivity analysis to estimate the model's sensitivity to parameter variation.

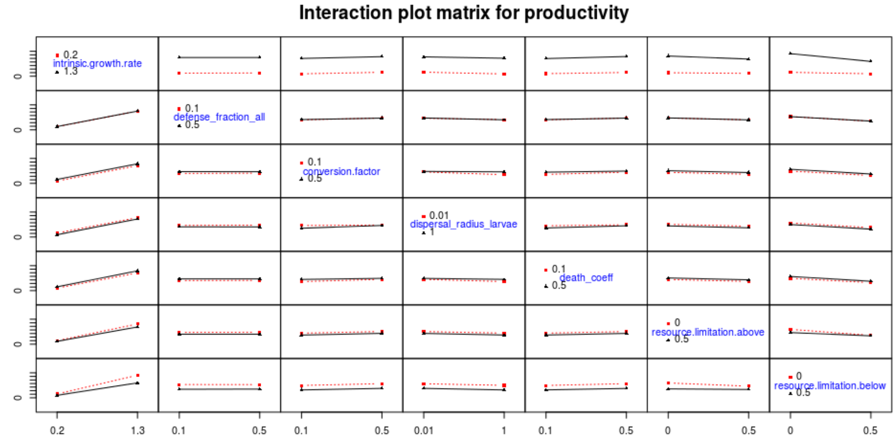


Figure ST25: Interaction effect plots (based on linear regression) on the productivity of plants. Top row: intrinsic growth rate of plants interacting with all other parameters (defense fraction (1,2), conversion factor of larvae (1,3), dispersal radius of larvae (1,4), death coefficient of larvae (1,5) and resource limitation above (1,6) and below (1,7)). For the other rows the method is equivalent, only other parameters are used. Red dotted line: value of the intrinsic growth rate is 0.2, black solid line: value of the intrinsic growth rate is 1.3. Two parameter values (the extrema) are compared for each parameter (left and right value, e.g. for the defense fraction the results of value "0.1" is compared to the resulting productivity of value 0.5. Lines in parallel indicate no interaction effect. Strongest interaction effect in this plot: intrinsic-growth-rate ⇔ resource-limitation-below.

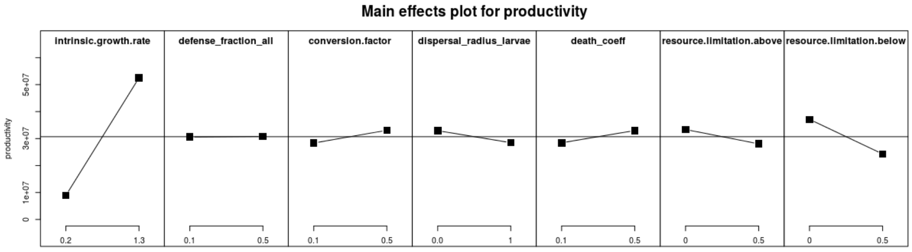


Figure ST26: The effect of variation of 7 parameters onto the plant's overall productivity. The strongest effect is reached by changing the intrinsic growth rate and the resource limitation below.

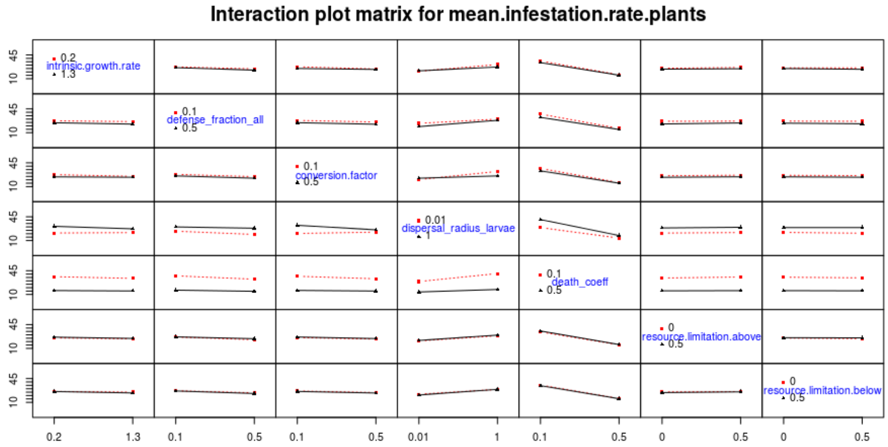


Figure ST27: Interaction effect plots (based on linear regression) on the infestation rate of plants. Top row: intrinsic growth rate of plants interacting with all other parameters (defense fraction (1,2), conversion factor of larvae (1,3), dispersal radius of larvae (1,4), death coefficient of larvae (1,5) and resource limitation above (1,6) and below (1,7)). For the other rows the method is equivalent, only other parameters are used. Red dotted line: value of the intrinsic growth rate is 0.2, black solid line: value of the intrinsic growth rate is 1.3. Two parameter values (the extrema) are compared for each parameter (left and right value, e.g. for the defense fraction the results of value "0.1" is compared to the resulting infestation rate of value 0.5. Lines in parallel indicate no interaction effect. Noticeable interaction effects on the plant's infestation rate: conversion factor larvae ⇔ dispersal radius larvae and dispersal radius larvae ⇔ death coefficient larvae.

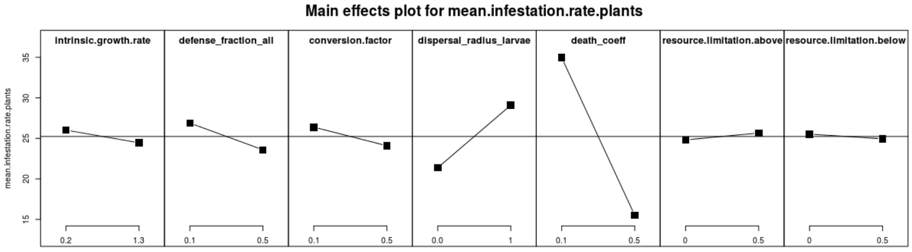


Figure ST28: The effect of variation of 7 parameters onto the plant's infestation rate. The strongest effect is reached by changing the death coefficient of larvae. This is logical, as when mmore larvae die less larvae do infest plants. As well the dispersal radius of larvae is important: the further larvae can go the more plants get infested.

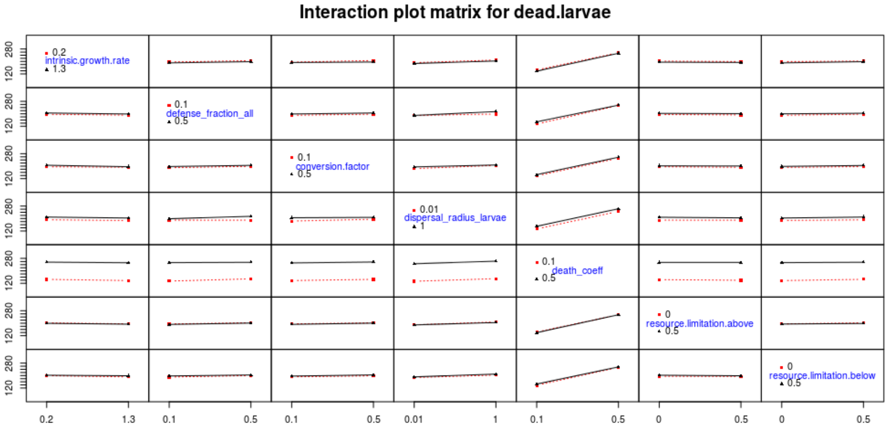


Figure ST29: Interaction effect plots (based on linear regression) on the number of dead larvae. Top row: intrinsic growth rate of plants interacting with all other parameters (defense fraction (1,2), conversion factor of larvae (1,3), dispersal radius of larvae (1,4), death coefficient of larvae (1,5) and resource limitation above (1,6) and below (1,7)). Red dotted line: value of the intrinsic growth rate is 0.2, black solid line: value of the intrinsic growth rate is 1.3. Two parameter values (the extrema) are compared for each parameter (left and right value, e.g. for the defense fraction the results of value "0.1" is compared to the resulting number of dead larvae of value 0.5. Lines in parallel indicate no interaction effect. No remarkable interaction effects on the number of dead larvae have been shown.

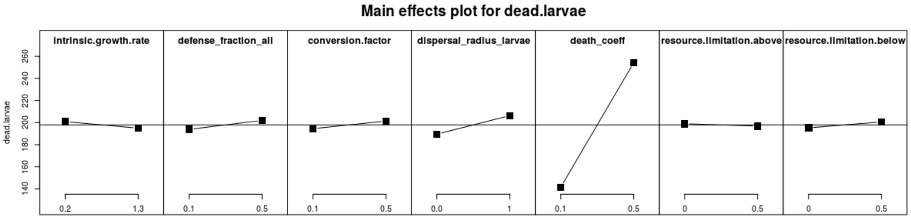


Figure ST30: The effect of variation of 7 parameters onto the number of dead larvae (300 larvae are initialized in one simulation). Here, the death coefficient has the strongest effect, also as well the dispersal radius of larvae has a considerable effect, that larvae which may walk further are more likely to die.

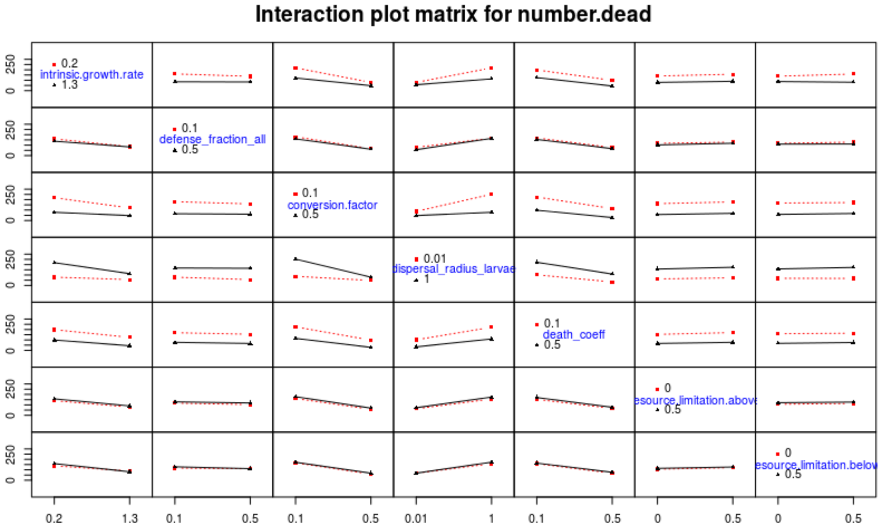


Figure ST31: Interaction effect plots (based on linear regression) on the number of dead plants. Top row: intrinsic growth rate of plants interacting with all other parameters (defense fraction (1,2), conversion factor of larvae (1,3), dispersal radius of larvae (1,4), death coefficient of larvae (1,5) and resource limitation above (1,6) and below (1,7)). Red dotted line: value of the intrinsic growth rate is 0.2, black solid line: value of the intrinsic growth rate is 1.3. Two parameter values (the extrema) are compared for each parameter (left and right value, e.g. for the defense fraction the results of value “0.1” is compared to the resulting number of dead plants of value 0.5. Lines in parallel indicate no interaction effect. Some strong interaction effects on the number of dead plants are present: intrinsic-growth rate ⇔ conversion factor, intrinsic-growth rate ⇔ dispersal radius larvae, dispersal-radius larvae ⇔ conversion factor, death coefficient larvae ⇔ conversion factor larvae and dispersal radius larvae ⇔ death coefficient larvae.

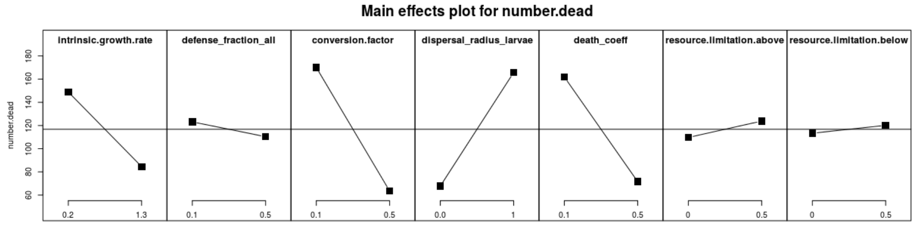


Figure ST32: The effect of variation of 7 parameters onto the number of dead plants. Here many parameters do strongly influence the plant mortality: the intrinsic growth rate, thus if plants grow faster, the plant mortality shrinks, the conversion factor of larvae: if the conversion factor is lower, larvae are forced to consume more plant material and more plants die, the dispersal radius of larvae is important: if larvae may walk further, more plants die and the death coefficient is proportional to plant survivability: if more larvae die more plants can survive.

Full Factorial Analysis

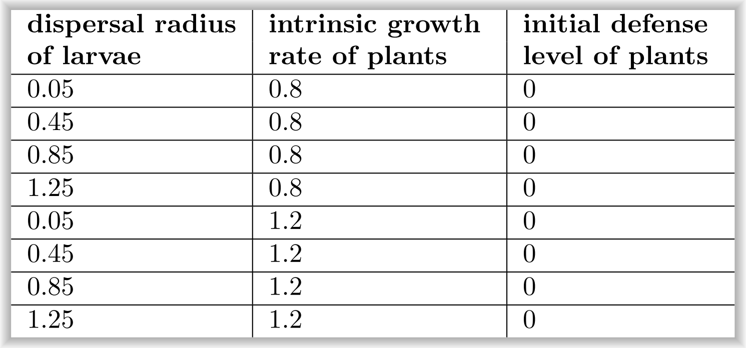
Because of field work experiences we had estimates about larval growth rates and plant mass gain and densities, however some variables remained unknown or were just estimated for our model. To get a more exact idea to which degree variation of those variables influenced the outcomes (like: how many larvae might die within one model run etc.) we varied the unknown parameters in a way that several sets of parameter combinations were produced. For these we checked whether the modelling results were within a realistic range which matched our field observations. This procedure is called "Full Factorial Analysis". Parameter sets resulting in non-realistic values were discarded. 10 repetitions per parameter combination were run.

parameter	**min value**	step size	**max value**
dispersal radius of larvae	0.3 m	2.7 m	8.3 m
intrinsic growth rate of plants	0.4	0.4	1.2
initial defense level of plants	0	20%	40%

Table ST5: **Parameters with unknown exact values:** We varied the unknown parameters in several steps to see whether the model's outcomes where still realistic, independent of our estimate.

range	**productivity**	**dead.larvae**	**dead.plants**	**infestation rate**
Minimum	100 g per plant	100	50	10 %
Maximum	450 g per plant	250	200	40 %

Table ST6: **Calibration ranges:** Output values which are used in the Full factorial analysis to estimate the accuracy of the model's predictions for multiple parameter combinations. The calibration criteria were set to fulfill field observations.



dispersal radius of larvae	intrinsic growth rate of plants	initial defense level of plants
0.05	0.8	0
0.45	0.8	0
0.85	0.8	0
1.25	0.8	0
0.05	1.2	0
0.45	1.2	0
0.85	1.2	0
1.25	1.2	0

Table ST7: **Results of the Full Factorial Analysis** Parameter sets which fulfilled all calibration criteria.

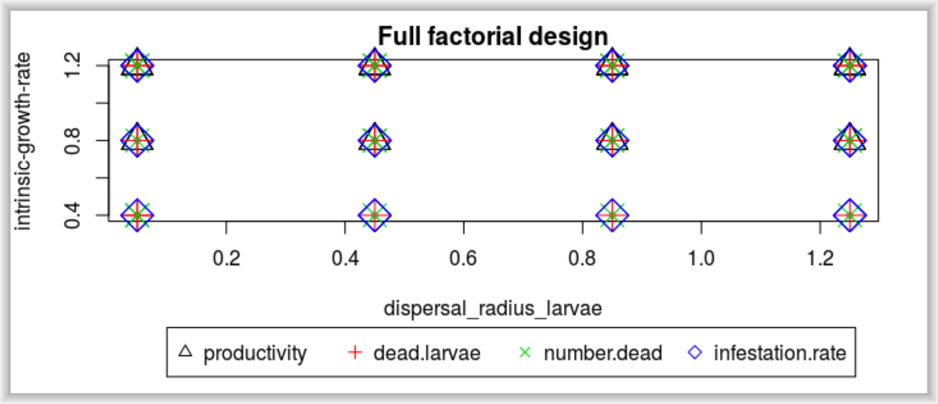


Figure ST33: Results of the full factorial design using categorical evaluation criteria. Grey points symbolize the tested parameter combinations, and the four different symbols show whether the evaluation criteria were met (black triangle: productivity criterion, red cross: dead larvae criterion, green x: dead plants criterion and blue diamond: infestation rate criterion). Here, the parameters “intrinsic growth rate” and “dispersal radius larvae” were varied and all combinations tested.

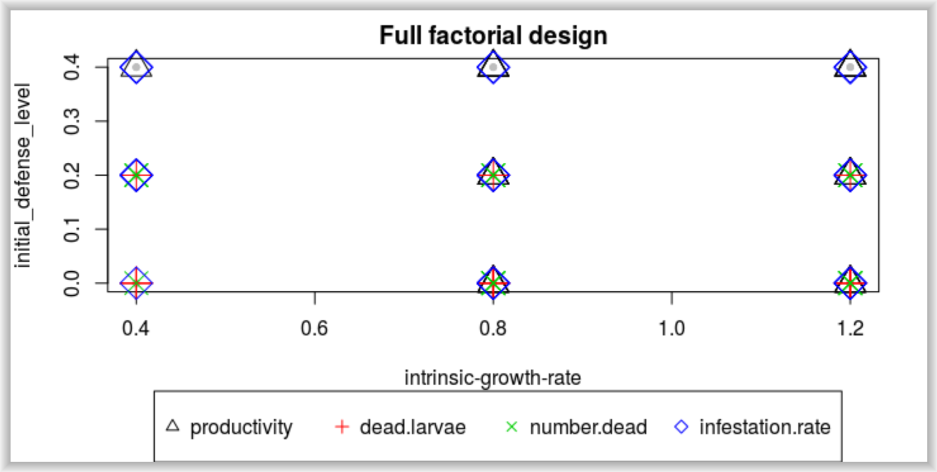


Figure ST34: Results of the full factorial design using categorical evaluation criteria. Grey points symbolize the tested parameter combinations, and the four different symbols show whether the evaluation criteria were met (black triangle: productivity criterion, red cross: dead larvae criterion, green x: dead plants criterion and blue diamond: infestation rate criterion). Here, the parameters "intrinsic growth rate" and "initial defense level of plants" were varied and all combinations tested.

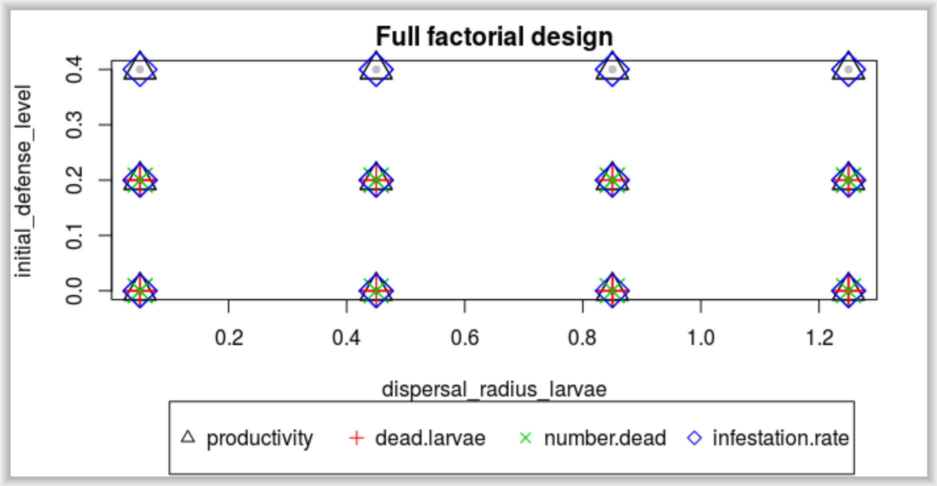


Figure ST35: Results of the full factorial design using categorical evaluation criteria. Grey points symbolize the tested parameter combinations, and the four different symbols show whether the evaluation criteria were met (black triangle: productivity criterion, red cross: dead larvae criterion, green x: dead plants criterion and blue diamond: infestation rate criterion). Here, the parameters "dispersal radius larvae" and "initial defense level of plants" were varied and all combinations tested.

Results of FFA:
To fulfill all criteria, the following parameter restrictions will be made:

- intrinsic growth rate of plants must be >= 0.8
- initial defense level must be <= 0.2

The dispersal radius of larvae is not so important, for very small, medium and large distances the outcomes fulfilled all calibration ranges.

0.8 Model output corroboration

This TRACE element provides supporting information on: How model predictions compare to independent data and patterns that were not used, and preferably not even known, while the model was developed, parameterized, and verified. By documenting model output corroboration, model users learn about evidence which, in addition to model output verification, indicates that the model is structurally realistic so that its predictions can be trusted to some degree.

In order to corroborate the findings of our model, we have collected plant samples of 60 plants of different native *N. attenuata* populations during the field season 2015. We induced half of the plants by wounding them with a pattern wheel and applying oral secretions of *M. sexta* into the wound. The other half of the plants we used as control. For both groups we collected leaf samples before, 1h after, 4h after, 24 h after, 48h after, 72 h after and 96 h after elicitation. Leaf samples were analyzed by a non-targeted analysis for secondary metabolites. Currently, analyses are running. When finished they will enable us to see to which amount the delay times of plants vary within a natural population and whether or not there is an optimal value which corresponds to the results of the TIMELY model.

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