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Fitness components in the relationship between *Rhopalapion longirostre* (Olivier, 1807) (Insecta: Coleoptera: Apionidae) and *Alcea rosea* (Linnaeus, 1758) (Malvaceae). Analysis of infestation balance of a herbivorous weevil and its host plant

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Abstract. Multivariate statistics (principal components analysis, path analysis) were used to investigate fitness components of the interactions between the weevil *Rhopalapion longirostre* (Olivier, 1807), Apionidae, Coleoptera and its host plant *Alcea rosea* (Linnaeus, 1758), Malvaceae. We focused on the activities of the larvae such as the choice of seeds for consumption, the preparation of seed chambers as a site for pupation, as well as the construction of escape holes through which the adults later emerge.

The analyses revealed that the optimal conditions for successful development of the weevils depended on the availability of seed capsules characterized by a high number of well developed seeds, few undeveloped and few spoiled seeds. The high number of larvae, pupae and not emerged adults found in the seed capsules corresponds with the successful emergence of adults. Egg deposition by the females in appropriate flower buds of the host plant, together with larval contribution to overall reproductive success are important fitness components in the life cycle of the weevil. The reproductive success of the plant is partly guaranteed by a high number of seed chambers, many well developed seeds, many not infested seeds, and few undeveloped seeds. Furthermore, the extended blooming period of the host plant serves as an escape strategy by developing buds early or late in the season that cannot become entangled in the life cycle of *R. longirostre*.

Key words. *Alcea rosea,* Malvaceae*, Rhopalapion longirostre,* Apionidae; Host plant-parasite interaction, infestation balance, larval contribution, path analysis, PCA, reproductive success

INTRODUCTION

Life history theory provides a framework for analyzing the contribution of major features of the life cycle to reproductive fitness and seeks to place these features in an evolutionary context (Roff 1992; Stearns 1992; Stearns & Hoekstra 2000). The life history events of most of the 62,000 described species of weevils or snout beetles (Curculionoidea, Coleoptera) (Alonso-Zarazaga & Lyal 1999), and their immature stages are incompletely documented and more or less episodic. As holometabolous insects, weevils undergo a complete metamorphosis which produces larval and adult stages each with their own life habits and physiological and behavioral traits thus enhancing the possibility for adaptation, specialization and evolution. The phenomenal diversity of weevils was discussed recently in evolutionary terms by Oberprieler et al. (2007). Considered as a group, weevils utilize nearly all parts of host plants from almost all plant taxa (Anderson 1995).

The larvae of most Apionidae, with about 1800 described species (Kuschel 1995), feed internally in stems, leaves, buds, galls, fruits or seeds of their specific host plants. The evolution of larval habits and host plant associations are taken for analysis of larval role in weevil's diversification (Marvaldi et al. 2002).

In this paper we examine several fitness components of the weevil *Rhopalapion longirostre* (Olivier, 1807) which is monophagous on its host plant *Alcea rosea* (Linnaeus, 1758), with special focus on larval contribution to reproductive success (Reavey & Lawton 1991). Seeds of *A.*

rosea, presumably derived from Anatolia (Hammer 1994), were brought to France in the 18th century. The beauty and popularity of this garden plant, known as the hollyhock, passe-rose, rose trémière, or Stockrose, was lovingly recorded in verse by King Ludwig XVI of France in the 18th century: L'Amour de moi s'y est éclosé / dedans un petit jardinet, / où croit la rose et le muguet / et aussi bien la *passe-rose*.

Today, the plant occurs in many parts of Europe, North and South America, mainly due to its cultivation in gardens. The development of the larvae of *R. longirostre* takes place in the buds and seeds of *A. rosea* and the expansion of the weevil's distribution range has proceeded in intimate association with that of the host plant (Perrin 1995; Gønget 1997).

Females of *R. longirostre* have a body size from 2.4 to 3.4 mm excluding the rostrum (Freude et al. 1983). They employ their extremely elongated rostrum (average size 2.2 mm, standard deviation +/- 0.2 mm) (Wilhelm 2004), to bore holes into developing buds of *A. rosea*. The plant attains a height of 1 to 3 meters and can persist for more than two years. The buds appear in a long inflorescence composed of 30 to 50 buds. Every bud is surrounded by thick inner and outer sepals which have stiff grey-green hairs (Adler et al. 1994). After, or even during copulation, with the male sitting on her back, the female bores a channel into a selected bud using her rostrum. She turns around and inserts her ovipositor into the bore channel penetrating the outer and inner sepals of the plant to lay three to four eggs into it. Female weevils prefer to bore into buds located in the middle of the inflorescence. These buds will usually not bloom in the following 2 weeks thus allowing enough time for the development of the larvae. If a female is among the first to bore a hole into the bud, she chooses the side of the plant which receives the morning sunlight. If several females have oviposited in the same bud, the boring channels are widely spaced from each other, presumably to avoid competition among the larvae for food. The first instar larvae are legless, as most of weevils larvae have lost the development of legs (Crowson 1955; Stehr 1991; Marvaldi 1997; Farrell 1998), about 0.4 mm long, and they hatch after 3 to 4 days. If the bore channel is in the upper part of the bud then the larvae of the first stage consume the surrounding pollen grains. However, if the bore channel is located at the bottom side of the bud, then the larvae feed only on the soft tissue of the bud. All larvae bore feeding channels toward the direction of the ovules in the developing seed capsule, using their strong mandibles to bite and chew. By the time the bud has blossomed and before it falls off, all larvae must have migrated below the ovary. Within a few days the upper part of the blossom wilts and falls to the ground. When the seeds of *A. rosea* are fully developed, each larva tries to enter a seed chamber from the bottom of the seed capsule and consume the entire contents of the seed from within. Prior to pupation, the last instar bores a hole on the broadest side of the seed chamber to later escape when it reaches the adult stage. The escape hole is filled in with a secretion produced by the larva to protect the pupa and the developing weevil against outside influences, e.g. wetness and sunlight. Pupation lasts between 2 to 3 weeks depending on weather conditions. The darkening of the adult weevil's exoskeleton is visible through the transparent membrane of the pupa beginning at the apex of the rostrum and the elytra. The adult beetle slowly emerges from the dry and stiff seed chamber over a period of several hours using its rostrum to get out through the escape hole. A duration of 8 to 10 weeks is required from egg deposition to the emergence of adult weevils.

Additional details on the biology of *R. longirostre* are found in Dieckmann (1977), Behne (1998), Pupier (1997), Sprick et al. (2002) and Wilhelm (2004).

The interaction between the host plant and the weevil is reflected in a successful joint expansion in Europe over the last twenty years. To demonstrate this a null hypothesis was postulated which denied significant interactions between the life cycles of host plant and weevil. To test null hypothesis in detail, we posed the following questions:

(1) What parameters lead to optimal prerequisites for successful development of weevils in seed capsules? In particular, what are the conditions of capsules which contain larvae, pupae and immature adults? Do these conditions correspond with successful emergence of adults?

(2) Which counter strategies are taken by the host plant to survive or to ensure its successful growth and reproduction?

(3) How effective is larval contribution to the overall reproductive success of the weevil?

MATERIALS AND METHODS

To address these questions we examined dry seed capsules collected from 20 *Alcea rosea* plants growing on the terrace at the University of Vienna, Althanstrasse 14, in the first week of Nov. 2006. For measurements we examined only dry capsules (n=205) having a complete set of seed chambers. Seed capsules, which were incomplete because some seed chambers had already fallen to the ground, were not investigated. The plants, 1-20, contained the following number of seed capsules: 7, 10, 11, 4, 5, 6, 21, 4, 16, 8, 6, 11, 13, 24, 15, 13, 7, 9, 6, 9.

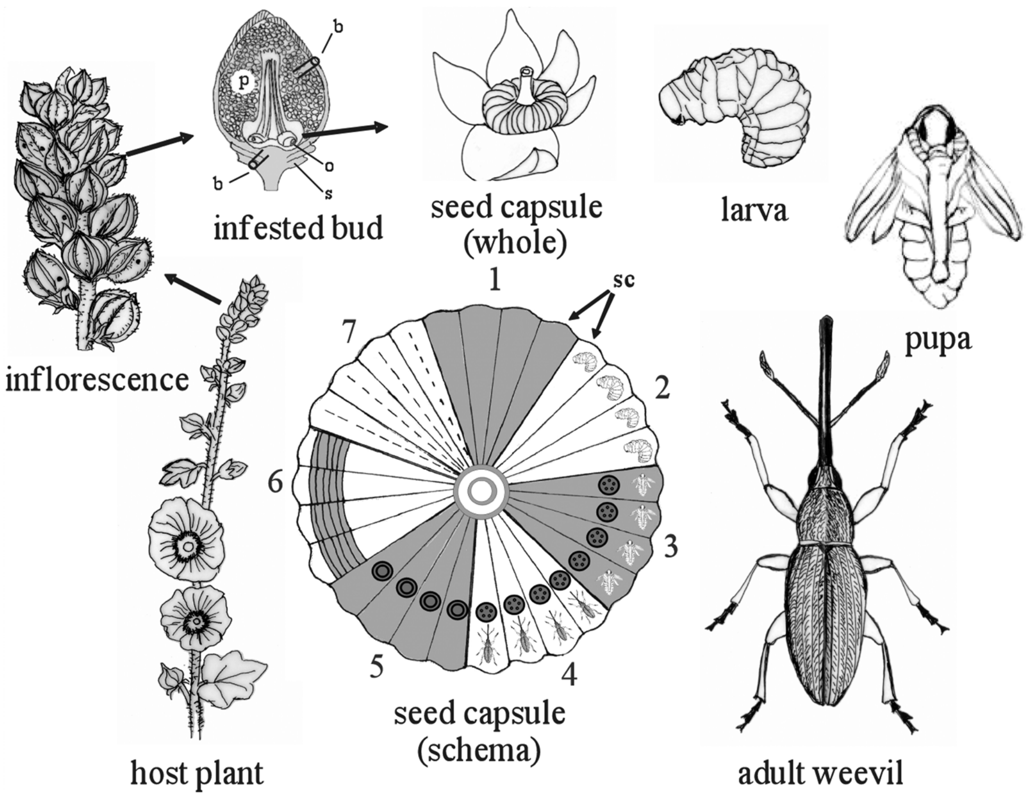

Fig. 1. Brief outline of life history stages of the weevil *Rhopalapion longirostre* on its host plant, *Alcea rosea*. Schema showing various conditions of a seed capsule: seed chambers with 1) fully developed seeds, 2) seeds infested with larvae, 3) pupae and closed escape holes, 4) not emerged adults and closed escape holes, 5) escape holes, 6) spoiled seeds, 7) undeveloped seeds. Abbreviations of infested bud: b bore channel, o ovules, p pollen, s sepal; sc seed chamber.

The diameter of each capsule was measured 5 times to cancel out error. The number of dry seeds per capsule and the condition of every capsule were documented. The seed chambers were opened under a dissecting microscope to determine the inner condition. The infested condition was indicated by the presence of larvae, pupae or not emerged adults, or empty seed chambers with open escape holes. The uninfested condition was indicated by the presence of seed chambers which are fully developed, undeveloped or spoiled. In the following, the data were analyzed thoroughly by multivariate statistics to estimate the importance of animal and plant related factors and their interactions on the reproductive fitness of weevil and plant.

For the following case study nine character variables of the seed capsules were measured for the statistical analyses.

Diameter of capsules in mm: **diam** (plant related: **PR**)

Number of seed chambers per capsule: **nrsc** (**PR**)

Number of open beetle escape holes per capsule: **nreh** (animal related: **AR**), i.e. estimate of the number of beetles which have successfully escaped.

Number of dead larvae per capsule: **nrla** (**AR**)

Number of dead pupae per capsule: **nrpu** (**AR**)

Number of dead, not emerged adults per capsule: **nrne** (**AR**)

Number of fully developed seeds per capsule: **nrds** (**PR**)

Number of undeveloped seeds per capsule: **nrus** (**PR**)

Number of spoiled seeds per capsule: **nrss** (**PR**)

In preparation of multivariate analyses, the original data were transformed as follows: The first variate was logarithmically transformed (natural log). In order to fulfill the

Table 1. Correlation matrix of transformed variates and significant levels: *** = highest significant P-value ≤ 0.001, ** = highly significant P-value ≤ 0.01, * = significant P-value ≤ 0.05. Bonferroni corrected. Variates: **diam** diameter of seed capsule, **nrsc** number of seed capsules, **nreh** number of escape holes, **nrla** number of larvae, **nrpu** number of pupae, **nrne** number of not emerged adults, **nrds** number of fully developed seeds, **nrus** number of undeveloped seeds, **nrss** number of spoiled seeds. The significance of correlation coefficients was determined by a random permutational test (Nemeschkal 1999).

	diam	nrsc	nreh	nrla	nrpu	nrne	nrds	nrus	nrss
diam	1.0000								
nrsc	0.075632	1.0000							
nreh	0.057043	0.167632*	1.0000						
nrla	0.032532	0.293509***	- 0.135968	1.0000					
nrpu	0.013449	0.280319***	- 0.044457	0.538041***	1.0000				
nrne	- 0.07478	0.129488	0.128997	0.161018	0.212016***	1.0000			
nrds	0.036070	- 0.046134	- 0.247253***	- 0.304999***	- 0.31991***	- 2.242189***	1.0000		
nrus	0.000937	0.163294	- 0.257581***	- 0.028526	- 0.088356	- 0.046297	- 0.306250***	1.0000	
nrss	- 0.001917	0.254107***	- 0.267961***	0.096727	0.208869**	0.088184	- 0.402289***	0.165270*	1.0000

Table 2. Factor matrix, axis 1 to 7, and significant axes of loadings. *** = highest significant, P-value ≤ 0.001, ** = highly significant P-value ≤ 0.01, * = significant P-value ≤ 0.05, ns= no significance P-value > 0.05, eigenvalues, and percentages of the total variance. The significant levels were determined by a random permutational test (NEMESCHKAL 1999, 10000 iterations). Potential win for animal and plant. + A, + P; potential loss: - A, - P. Abbreviations of axes: **SUPLA** for ´successful plant (seed) production´; **SUAN for** ´successful animal production´; **SUMIX** for ´integration of the two trends, successful animal and plant production´; **LIFA** for ´late infestation axis´ and **DASA** for ´damaged seed and animal axis´.

Factor axes Abbreviations	F1 **SUPLA**	F2 **SUAN**	F3 **SUMIX**	F4 **LIFA**	F5 **DASA**	F6 **n.n.**	F7 **n.n.**
var 3 nreh	- 0.083229ns	- 0.723916***	0.568846***	- 0.237805**	0.174577ns	- 0.024872ns	0.240388***
var 4 nrla	0.680778***	- 0.168066*	- 0.463831***	- 0.297076***	- 0.174923ns	0.394877***	0.135561ns
var 5 nrpu	0.726492***	- 0.245330***	- 0.380804***	- 0.114225ns	0.074140ns	- 0.498353***	0.010510ns
var 6 nrne	0.430808***	- 0.379749***	0.211681***	0.631854***	- 0.475213***	0.012534ns	0.012746ns
var 7 nrds	- 0.743359***	0.012772ns	- 0.532765***	0.217806**	- 0.116773ns	- 0.103023ns	0.302864***
var 8 nrus	0.217084**	0.687623***	0.397469***	- 0.274868***	- 0.443069***	- 0.147826ns	0.168382ns
var9 nrss	0.559095***	0.458271***	0.118490ns	0.384199***	0.525592***	0.073805ns	0.184544**
Eigenvalues	2.096067	1.439697	1.184405	0.827693	0.778592	0.442973	0.230572
percentage of total variance	29.94	20.57	16.92	11.82	11.12	6.33	3.29
animal win/loss	-A	+A	+A	+A	-A		
Plant win/loss	+P	-P	+P	+P	-P		

linearity requirement, variates 2 to 9 were substituted by their square roots. Finally, a product moment correlation matrix was calculated between all variates over the 205 capsules (Table 1).

The correlation structure of variates 3 to 9 was then analyzed by a principal components analysis (PCA, for details see Morrison 2004). An orthogonalization was reached via PCA by calculating a full analysis and extracting all possible, i.e. 7, axes (Stat. Graphics – Version 7.3 for MS DOS). The eigenvectors were scaled according to eigenvalues leading to a factor matrix (Table 2). The significance of loadings on principle axes was determined by a random permutational test, 10000 random permutations each, (Nemeschkal 1999). Note that the original variates are represented on factor axes by their loadings. A loading is the product moment correlation of an original variate with the factor axis. For details and further statistical explanation, see Morrison (2004).

Path analysis**,** a special type of regression analysis, (Stat. Graphics – Version 7.3 for MS DOS) was applied to represent the complicated animal-plant relations in diagrammatic form (Path diagrams, Figs. 2 and 3). In addition, path analysis has the advantageous option to focus dependencies either on plant or for animal variables (for theoretical details on path analysis, see Sokal & Rohlf (1995).

For reasons of simplicity, our analyses are mainly based on procedures of the general linear model. In order to test whether a loss of information in the data structure has occurred due to data transformations, we calculated, in parallel log-linear analyses with the non-transformed data (see Appendix).

RESULTS

Of the 205 examined seed capsules, each contained an average of 35 individual seed chambers (ll 34.7758, ul 35.6798; ll: lower limit, ul: upper limit of 95% confidence of average). Of the 35 seeds chambers, an average of 13 (ll 11.6393, ul 13.4734) were fully developed, inferring the reproductive success of the host plant, 7 (ll 6.0002, ul 7.6396) had open escape holes, inferring the potential reproductive success of the weevils. The remaining 15 seed chambers (on average per capsule) were either infested (documented by dead larvae, pupae or not emerged adults), undeveloped or spoiled by mold or mites.

The results from the PCA and the path analyses reveal detailed information on the complex system of interactions between weevil and host plant. For the sake of simplification, the diameter and number of seed chambers were omitted from the PCA and path analyses since the simple correlation between the diameter (diam) and the remaining variates are not significant (Table 1) and since the number of seed chambers (nrsc) is a summation of variates 3 to 9.

A total of 7 axes are significant (Table 2). Factor axes 1 to 5 are the most important, accounting for over 90% of the total variance.

Results summarized for the five most important factor axes:

F1 represents nearly 30% of total variance. Positive and negative signs of the loadings indicate the reverse proportionality in the variational trends: nrla, nrpu, nrne, nrss (positive and very highly significant) and nrus (highly significant) versus nrds (negative and very highly significant).

F2 represents 21% of total variance. Cumulatively, the axes F1 and F2 explain half of the total variance. The loadings are contrasted to each other by their signs (+/-); i.e. nrus and nrss (very highly significant) and with positive signs, versus nreh, nrpu, nrne (very highly significant) and nrla (significant), both with negative signs.

F3 explains 17% of the total variance, and by accumulation of axis F1 to F3 more than two thirds of total variance are explained. The signs of loadings, nreh, nrne and nrus (very highly significant) contrast with those of nrla and nrpu (very highly significant).

F4 represents about 12% of total variance. Axes F1 to F4 cumulatively explain more than three quarters of total variance. The loadings are grouped by positive signs for nrne, nrss (very highly significant) and nrds (highly significant) and negative signs for nrla, nrus (very highly significant) and nreh (highly significant).

F5 explains 11% of total variance. The positive sign of the loading nrss (very highly significant) contrasts with those of nrne and nrus (very highly significant).

Factor axes 6 and 7 together provide 9% of total information and will not be further considered in this study.

Path Analyses

Two path analyses were conducted to focus on the main influences affecting the animal's or plant's life cycle. Variables 3 to 9 served as the basis for both path analyses. The first analysis determines the influence of variables 4 to 9 on variable 3 (nreh: number of escape holes per capsule, AR = animal related) (Fig. 2). In the second analysis variable 7 (nrds: number of fully developed seeds per capsule, PR = plant related) the variables 3 to 6, 8 and 9 take influence on variable 7 (Fig. 3).

Path Analysis I (Fig. 2): Since variable 3, nreh, number of escape holes per capsule, may be an indicator of fitness for the weevil, it was subjected to a multivariate path analysis. Simple arrows indicate the directions of influences of the predictors on criterion (= direct paths), double arrows indicate the correlation between predictor variables. The strength of influence is given by the value of the path coefficient. The path coefficient is a standardized multiple regression coefficient (path coefficient = regression coefficient times standard deviation of predictor divided by the standard deviation of criterion). The path coefficient quantifies the amount of change in criterion when progressing in independent variables. There are 4 direct paths from the predictors to the criterion. The first direct path proceeds from var 4, nrla, to the criterion and is negative and highly significant. It infers that the more dead larvae per capsule, the fewer escape holes. The second direct path from var 7, nrds, to the criterion is negative and very highly significant: the more well developed seeds, the fewer open escape holes. The third direct path proceeds

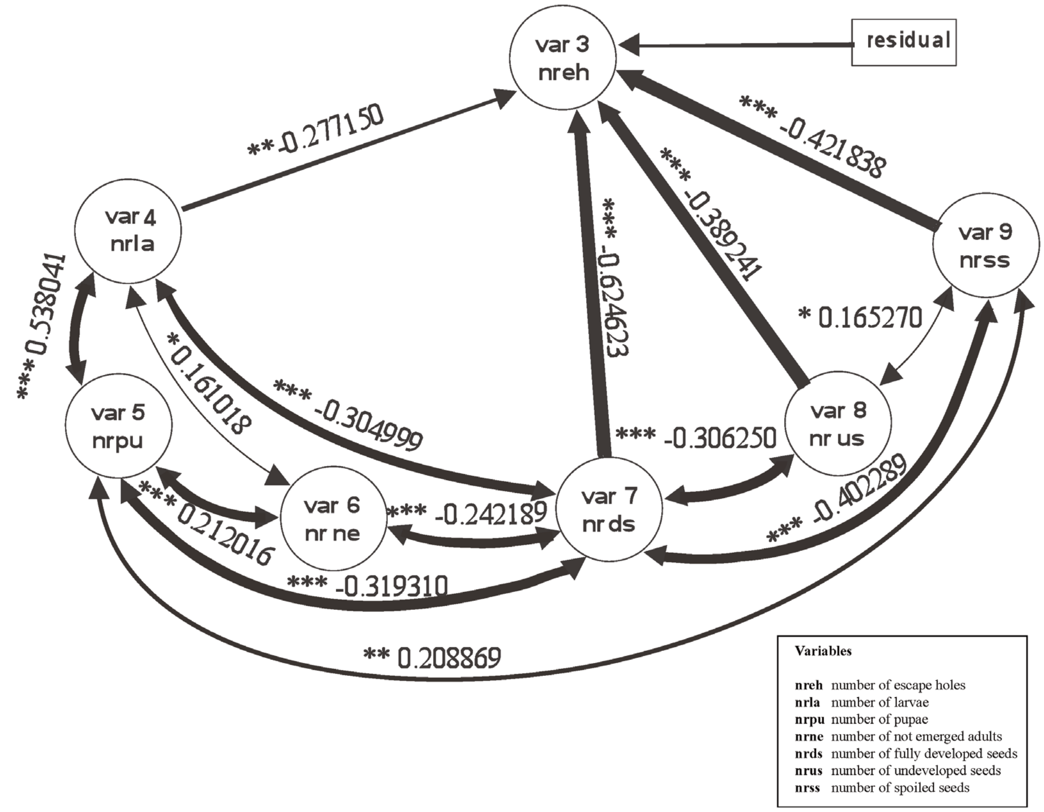

Fig. 2. Path diagram 1 showing 6 predictor variables and a residual effecting one criterion (var 3, nreh, number of escape holes, an animal related variate), $R^2 = 0.414525$. The path diagram explains 41.45% of the variance of the criterion. Only significant paths are shown. The significance of paths was determined by random permutational test; 10000 iterations each; Bonferroni Correction. Simple arrows indicate the direction of influences of the predictors to the criterion (= direct paths), double arrows indicate the correlation between predictor variables. The strength of influence is given by the value of the path coefficient.

from var 8, nrus, to the criterion and is negative and very highly significant: the more undeveloped seeds, the fewer escape holes. The fourth direct path proceeds from var 9, nrss, to the criterion and is negative and very highly significant: the more spoiled seeds per capsule, the fewer open escape holes. In addition, several indirect effects are apparent. Both direct and indirect paths in the diagram must be combined to indicate an overall influence on the criterion. The most important indirect path proceeds from nrla to the criterion via nrds, and is very highly significant: the more larvae, the fewer well developed seeds and the more escape holes (indirect effect: -0.304999 ∗ -0.624623 = 0.190509). Direct and indirect paths are mutually counteracting, therefore it is the summation of all effects that indicates the overall influence on the criterion, which in this case is negative: direct effect: nrla → nreh -0.277150 plus indirect effect: nrla → nrds → nreh 0.190509 results in an overall effect of -0.086641. A second indirect effect proceeds from nrla to the criterion via nrpu, nrne and nrds: the more larvae, the more pupae and the more not emerged adults means that there are fewer well developed seeds and more escape holes in the seed capsule (0.538041 ∗ 0.212016 ∗ -0.242189 ∗ -0.624623 = 0.017257). Another indirect effect between nrpu and nrds to the criterion is very highly significant: the more pupae, the fewer well developed seeds and the more escape holes (-0.31931∗ -0.624623 = 0.19945). The indirect effect which proceeds from nrds and nrus to the criterion is very highly significant: the more developed seeds, the fewer undeveloped seeds and the more escape holes (-0.30625) ∗ -0.389241 = 0.11921. The indirect effect which proceeds from nrds and nrss to the criterion is very highly significant: the more developed seeds, the fewer spoiled seeds and the more escape holes (-0.402289 ∗ -0.421838 = 0.1697). Overall, the indirect effects indicate a decrease of the negative influence from the direct paths to the criterion.

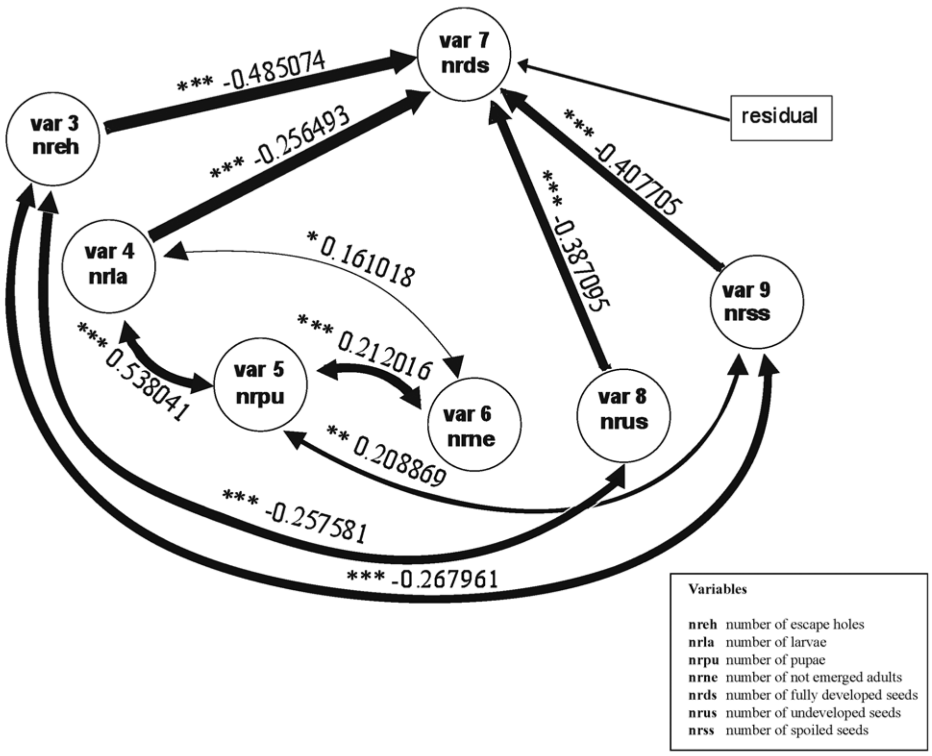

Fig. 3. Path diagram 2 showing 6 predictor variables and a residual effecting on criterion (var 7, nrds, plant related variate, number of fully developed seeds per seed capsule), $R^2 = 0.545329$. The path diagram explains 54.53% of the variance of the criterion. The significance of paths was determined by random permutational test, 10000 iterations each, Bonferroni Correction.

Path Analysis II (Fig. 3): The second path analysis, using the same variables as in the first analysis, was calculated to determine the influence of variables 3–6, 8 and 9 on variable 7, nrds, number of fully developed seeds per capsule, which is the most important plant related factor. Since variable 7 may be an indicator of fitness for the plant, it was subjected to a multivariate path analysis.

There are 4 direct paths from the predictors to the criterion. The first direct path proceeds from var 3, nreh, to the criterion and is negative and very highly significant: the more escape holes, the less fully developed seeds per capsule. The second direct path from var 4, nrla, to the criterion is also negative and very highly significant: the more larvae, the fewer well developed seeds. The third direct path, from var 8, nrus, to the criterion is negative and very highly significant: the more undeveloped seeds, the fewer developed seeds per capsule. The fourth direct path, from var 9, nrss, to the criterion is also negative and very highly significant: the more spoiled seeds, the fewer well developed seeds.

There are several indirect effects between the predictors and the criterion. The indirect effect proceeds from var 3 and var 8 to the criterion and is very highly significant: the more escape holes, the less undeveloped seeds and the more fully developed seeds ($-0.257581 * -0.387095 = 0.09971$). The indirect effect between nreh and the criterion, via nrss, is very highly significant: the more escape holes, the fewer spoiled seeds and the more fully developed seeds ($-0.267961 * -0.407705 = 0.10925$). Both paths show a decrease of the negative influence of escape holes on the criterion. Another indirect effect from nrne, via nrpu, via nrla to the criterion is very highly significant: the more not emerged adults, the more pupae and the more larvae, the fewer well developed seeds will be found ($0.212016 * 0.538041 * -0.256493 = -029259$). This indirect effect shows a negative influence on the criterion.

In summary, both the PCA and the path analyses explain animal - plant interactions; path diagram 1 focuses on the animal, while path diagram 2 centers on the plant.

Discussion

The relationships between insects and plants are of utmost importance for terrestrial global ecology. Many of these relationships involve specialized herbivorous insects and their host plants on which the females oviposit and on which the larvae feed (Schoonhoven et al. 1998). The choice of an oviposition site is instrumental for the fitness of many weevils (Messina 2004). In a case study approach we investigated fitness components of the interactions between the weevil, *R. longirostre* and its host plant, *A. rosea*.

The life cycles of the weevil and its host plant must be considered in connection with each other. With the help of multivariate statistics it was possible to isolate several main factors which contribute toward the survival of the host plant, as well as the successful development of the weevil. The lengthy inflorescence of *A. rosea* may contain of more than 30 buds in different development stages ranging from fully developed buds at the bottom, which will blossom soon, to immature tiny buds at the top. Host plant preferences of the female weevils for oviposition sites are buds located in the middle of the inflorescence. Exactly these offsetting development stages of the buds are reflected in the PCA (Table 2). In summary, the PCA provides an overview of the interaction system between the plant and the weevil.

Factor axis 1, which is the most important one, is interpreted as the axis which reflects successful seed development: The variate of final production outcome in the plant, i.e. nrds, number of developed seeds, is contrasted to the counteracting variables of numbers of undeveloped seeds, nrus, and spoiled seeds, nrss, and indicators of weevil development, nrla, nrpu and nrne. The axis is to be read as follows: the more developed seeds, the less dead larvae, pupae and not emerged adults in the capsules but also the less undeveloped and spoiled seeds. In contrast to this interpretation, axis 1 could be read as: the more dead larvae, pupae and not emerged adults inside the capsules and the more undeveloped and spoiled seeds, the less developed seeds. Note that the variable of plant final success, nrds, is exclusively represented on axis 1 but not the variable of the animal final success, nreh. Consequently, we assigned to axis 1 the abbreviation SUPLA for "successful plant" (i.e. seed) production.

The next most important axis, factor axis 2, is to be interpreted as mainly reflecting weevil development and production. The variate of final production outcome in the animal, i.e. nreh, number of escape holes, is combined in variation with the variates of animal development, i.e. nrla, nrpu, and nrne. Whereas the first variate, nreh, signifies production success, the remaining variates nrla, nrpu and nrne stand for the unsuccessful weevil development in the host plant. However, all the animal related variates are contrasted to plant variates which stand for unsuccessful seed development, i.e. nrus and nrss – undeveloped and spoiled seeds. The axis loading read as: The less undeveloped and spoiled seeds, the more escape holes but also the more dead larvae, pupae and not emerged adults. Note that the variate of final production outcome in the plant, nrds, is not significantly represented on this axis. Consequently, we designate this axis SUAN, "successful animal" production, as a main infestation axis. Highly infested capsules were located in the middle of the plant's inflorescence. Early infestation of seed capsules guarantees high reproductive success for the weevil as shown by the high proportion of open escape holes. In cases where a high number of seeds were infested, the number of undeveloped and spoiled seeds was very low.

Factor axis 3 reflects the mutual interaction of weevils and plant production. In one group of variates the final production outcome of the animal, nreh, and the pre-developmental stage to that final outcome, i.e. nrne, are combined with the number of undeveloped seeds. In contrast, in the second group of variates, the final production outcome of the plant, nrds, is combined with the developmental variates of the weevil, nrla and nrpu. The axis loadings can be read in two different ways, both immediately indicating a competitive race between animal and plant to maximize their fitness (as shown for a seed predatory weevil and its host plant, Toju, Sota 2006). On the one hand, the more escape holes, as well as not emerged adults and undeveloped seeds in capsules, infer fewer developed seeds, as well as larvae and pupae. On the other hand, the more developed seeds, as well as larvae and pupae, infer fewer escape holes, not emerged adults and undeveloped seeds. There are two counter acting trends: One, early infestation by the weevil which is characterized by a high number of open escape holes and not emerged adults together with a high number of undeveloped seeds. Since larvae only infest well developed seeds, few seeds remain for plant development. The second trend concerns late infestation and incomplete weevil development. It is characterized by a high number of larvae and pupae together with a high number of developed seeds. The abbreviation SUMIX (successful animal and plant production) represents the integration of the two trends.

Factor axis 4 can be interpreted as late infestation. Both variates, nrds and nreh, are highly significant and contrast each other. In the one group of variates, nrds is combined with nrss and nrne. In another group, nreh is combined with nrla and nrus. The variate loadings are interpreted as the following: many developed seeds and many spoiled seeds infer few undeveloped seeds in capsules, while the presence of fewer escape holes and fewer larvae infer more not emerged adults. A high number of spoiled seeds indicates that adverse weather conditions, mostly in late summer, could have influenced negatively both seed and weevil development, and in particular, the escape of adult weevils. We abbreviate this axis LIFA, "late infestation axis".

Factor axis 5 is an axis representing damaged seeds and late developmental stages of the weevils. The variate, nrss, contrasts with nrus and nrne. The loadings are interpreted as follows: The presence of many spoiled seeds infers fewer undeveloped seeds and fewer not emerged adults, and vice versa. This axis is termed DASA, "damaged seed and animal axis".

The main important axes of the PCA provide a comprehensive explanation about the complex system of interaction between animal and plant.

The influences and trends of both path diagrams must be seen in the light of direct paths and indirect effects (see Figs. 2 and 3). Our null-hypothesis was falsified by the significant correspondence between the life cycle of host plant and weevil. In path diagram 1, all direct paths (without exceptions) from the different variates to the criterion have strong negative influences. The sequence of indirect effects between the number of larvae → number of well developed seeds → criterion, as well as the indirect influence nrla → nrpu → nrne → nrds → criterion, nreh, are very important. As larval feeding habits are highly conservative some of these habits are apparently irreversible, e.g. feeding on seeds (Marvaldi et al. 2002). Larvae of the last developmental stage choose well developed seeds to enter, to consume and to prepare the seed chamber for pupation and future escape. The sequence of indirect effects, nrds → nrus → criterion, or nrds → nrss → criterion, are also very important. For the performance of a larva, it is vital to find a well developed seed instead of an undeveloped or spoiled seed.

In path diagram 2, the 4 direct paths (without exceptions) have also strong negative influences on the criterion nrds. The indirect effects are advantageous for both, plant and animal production. The sequence, nrne → nrpu → nrla → criterion, indicates that the high number of infested seeds are based on a high number of formerly well developed seeds. The sequences, nreh → nrus → criterion, nrds, and nreh → nrss → nrds, infer that high numbers of escape holes and few undeveloped or spoiled seeds increase the number of well developed seeds. A plant in a healthy condition guarantees the performance of the weevils. The time of infestation is crucial for the weevils. Our analyses reveal that an infestation occurring late in the season involves a high likelihood of poor plant condition, as shown in the indirect effect nrpu → nrss → nreh.

In conclusion, we address the initial questions posed in the introduction.

1) What are the optimal conditions for the reproduction of weevils? The most important optimal condition for weevil reproduction is the timely presence of many well developed seeds.

2) What are the optimal conditions for the successful development of plants? The optimal conditions for the successful development of plants are few undeveloped and few spoiled seeds.

3) Is the contribution of larvae to the overall reproductive success of the weevils important?

Yes, in both path diagrams, the animal related variate, i.e. number of larvae, has a highly significant direct path to the first criterion, number of escape holes, as well as to the second criterion, well developed seeds. Obviously larvae benefit from capsules with a very high number of well developed seeds. Since the number of larvae has an exclusively direct impact on both criteria, this influence is interpreted as a high larval contribution to the reproductive success of *R. longirostre*.

Our results strongly support the view of an intricate relationship between the life cycles of *A. rosea* and *R. longirostre.* The plants' period of bud development lasts from April to October. The weevils utilize a window within this range to fulfill their development, beginning in May after hibernation in the soil and ending in September with the completion of their life cycle and the emergence of the next adult generation from the seed chambers. The time periods before and after weevil activity ensure enough opportunity for successful production of seeds.

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APPENDIX

Poisson regression analysis

Dependent variate: nreh

Regression coefficients (predictors):

beta- 0 (constant):	4.632211	< 0.0001	
beta- 1 (nrla):	-0.170142	0.0002	***
beta- 2 (nrpu):	-0.040228	0.4612	n.s.
beta- 3 (nrne):	0.040783	0.5624	n.s.
beta- 4 (nrds):	-0.405291	< 0.0001	***
beta- 5 (nrus):	-0.267374	< 0.0001	***
beta- 6 (nrss):	-0.258430	< 0.0001	***

global model, deviance: 530.341739, P-value = < 0.0001, ***

The significance was tested by random permutational tests (10000 iterations each).

Dependent variate: nrds

Regression coefficients (predictors):

beta- 0 (constant):	3.797212	< 0.0001	
beta- 1 (nreh):	-0.166088	< 0.0001	***
beta- 2 (nrla):	-0.098854	0.0072	**
beta- 3 (nrpu):	-0.061230	0.1904	n.s.
beta- 4 (nrne):	-0.079595	0.1760	n.s.
beta- 5 (nrus):	-0.158951	< 0.0001	***
beta- 6 (nrss):	-0.170892	< 0.0001	***

global model, deviance: 340.101076, P-value = < 0.0001 ***

The significance was tested by random permutational tests (10000 iterations each).

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