High-Throughput Phenotyping of Plant Resistance to Insects

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Abstract

Devastating insect pests and environment-malignant pesticides that are applied against them are still a major problem in agriculture and horticulture. In plant breeding and genomic research there is an increasing demand for efficient screening methods of plants. In this study we develop a high-throughput phenotyping system to screen plants for resistance to aphids by video-tracking of insect behaviour.

Introduction

Studying the behaviour of herbivorous insects is essential for the improvement of agricultural pest management. Devastating insect pests still cause a significant amount of yield loss and a reduced marketability of food and ornamental crops [1]. Environment-malignant and costly pesticides are widely applied to control pests and diseases. There is, however, an increasing urge to improve the sustainability in the food production chain. Host-plant resistance is one of the cornerstones of successful and environmentally benign pest management systems [1,2]. Its concept is to reduce the intrinsic plant susceptibility to insect damage and thereby minimizing the need to apply pesticides. To improve plant resistance, suitable cultivars need to be selected among hundreds of plant lines. Screening plants for resistance to insects is generally a costly exercise in terms of space, time and labour. Bioassays often require a lot of greenhouse space including outbreak prevention measurements, and will take several weeks, depending on the insect life cycle. Eventually such a screening is mainly a manual procedure, such as counting of offspring, monitoring the development time to adulthood or measuring insect weight [3,4]. Plant breeding companies therefore need to invest a significant amount of money into these activities. Also, plant researchers face the need for efficient large-scale screenings now the costs for genotyping have rapidly declined and next-generation sequencing has rendered a wealth of genomic information [5]. For genomic research the screening of numerous natural accessions of crop-related plants is often a necessity. Devastating pests and diseases only rarely occur in nature, which is due to the tremendous degree of natural variation in plant defense mechanisms [6,7]. Only a relatively small degree of such variation is contained in cultivated crop populations [8], but wild populations provide ample opportunities for discovering novel mechanisms responsible for resistance to insects. In order to identify genes involved in plant resistance to insects, genomic studies are merely interested in the mechanisms of resistance rather than solely the identification of resistant plants. An effective screening method should therefore be able to discriminate distinct plant mechanisms that reduce insect colonization and feeding (e.g. plant volatile cues, deterrent surface structures, or toxins in the internal plant tissue) [9] (Figure 1).

Video-tracking of minute animals, such as 2 mm sized aphids, is a challenge. It requires a different approach in the hard- and software setup compared with video-tracking of larger sized animals, particularly when insects and plants have indistinctive colours. Although the miniscule size is demanding for automated subject detection, video-tracking of insect behaviour has a lot to offer in terms of accuracy and high-throughput; assets that are hard to achieve simultaneously during manual observations.

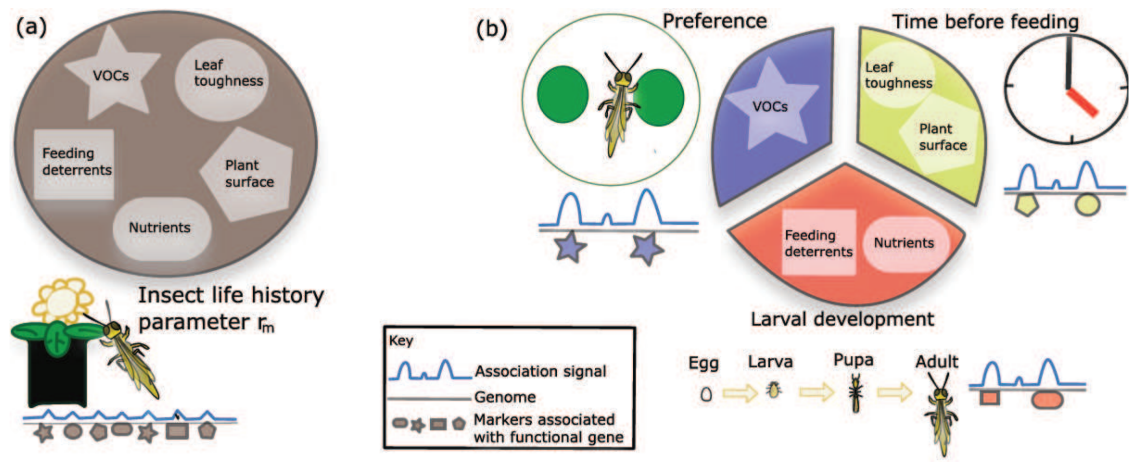

Figure 1. To find genes associated with resistance to insects, it is of particular importance to identify the mechanism(s) involved. This is illustrated in (a) where the life-history parameter rm (the intrinsic rate of population increase) of the insect shows which plant is resistant but seems to be associated with hundreds of genetic loci. A better approach to pinpoint the genes of importance, is to dissect the complex phenotype into component traits related to resistance mechanisms (b); e.g. insect preference (detection of repellent volatiles), time before the insect starts feeding (screening for the influence of leaf toughness and deterrent structures on the plant surface) and larval development (detection of, e.g. feeding deterrents, toxins and nutrient content) (figure adopted from [9]).

Aims

In this study we develop a high-throughput phenotyping system to screen for plant resistance to herbivorous insects. The system is based on video-tracking of insect behaviour and can be applied both for breeding purposes and genomic research with various insect and plant species. We study three components of insect behaviour: (1) host preference in a two-choice situation, (2) host acceptance (latency to the first feeding event and occurrence of dispersal events), and (3) feeding behaviour in general. We focus on the green peach aphid, *Myzus persicae*, a generalist phloem-feeding insect, that feeds on plants in more than 40 plant families and occurs virtually worldwide [10,11].

Methods and materials

The camera set up consists of arenas mounted on a backlight unit and two cameras with helicopter view for recording parallel sessions (Figure 2). The aphids are exposed to either a non-choice situation consisting of one leaf disc, or a two-choice situation consisting of two leaf discs of different plant types. We quantify two major behavioural components of aphid behaviour: (1) Probing behaviour (the activity when the aphid penetrates the plant tissue with its mouth parts in order to reach the phloem vessels) and (2) Non-feeding activities, such as the

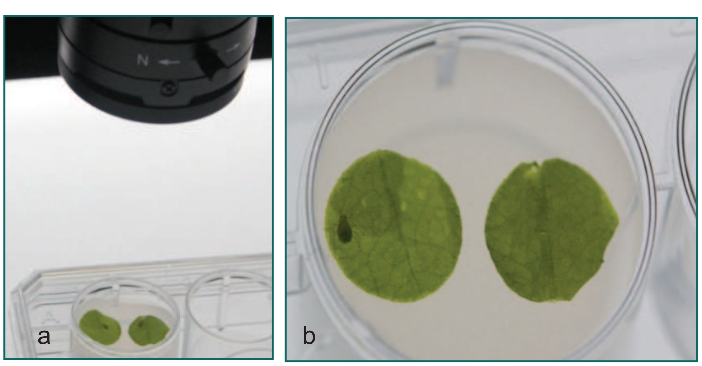

Figure 2. (a) Camera set-up. (b) Two-choice arena with a green peach aphid.

distance moved and the number of events when the aphid leaves the leaf disc. These parameters are registered over multiple time intervals, with multiple arenas observed simultaneously. We use the software EthoVision® XT for automated video tracking [12]. Data management and statistical analysis are performed with the program R [13]. Aphids exposed to resistant plants are expected to have a higher latency to the first feeding event and a higher frequency and duration of non-feeding activities compared with aphids exposed to susceptible plants.

With this video-tracking system we will screen 350 wild type plant accessions of mouse ear-cress, *Arabidopsis thaliana*. We will use the behavioural data of the aphids and the available genomic data of the Arabidopsis lines in a genome-wide association mapping study, in order to identify genes that are involved in resistance to aphids.

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