

LETTER

Self-recognition affects plant communication and defense

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Abstract

Animals have the ability to distinguish self from non-self, which has allowed them to evolve immune systems and, in some instances, to act preferentially towards individuals that are genetically identical or related. Self-recognition is less well known for plants, although recent work indicates that physically connected roots recognize self and reduce competitive interactions. Sagebrush uses volatile cues emitted by clipped branches of self or different neighbours to increase resistance to herbivory. Here, we show that plants that received volatile cues from genetically identical cuttings accumulated less natural damage than plants that received cues from non-self cuttings. Volatile communication is required to coordinate systemic processes such as induced resistance and plants respond more effectively to self than non-self cues. This self/non-self discrimination did not require physical contact and is a necessary first step towards possible kin recognition and kin selection.

Keywords

Artemisia tridentata, eavesdropping, induced resistance, kin selection, neighbour, non-self, plant behaviour, self, volatile cue.

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INTRODUCTION

The ability to distinguish between self and non-self is highly conserved and has been documented for many multicellular organisms, although it is not well known for plants (Klein 1982; Grosberg 1988). Self-recognition allows the possibility of preferentially reducing antagonistic interactions and increasing facilitative interactions with other ramets or tissues that are genetically identical (Waldman 1988). Clear demonstrations of self-recognition in plants are rare and the mechanisms involved are poorly understood. Some plants distinguish self from non-self and adjust their growth accordingly. For example, roots of *Ambrosia dumosa* inhibit the growth of roots of other individuals when they come into direct physical contact (Mahall & Callaway 1991, 1996). Contact between connected roots of the same individual failed to inhibit growth, although roots of separate cloned cuttings of an individual were not recognized as self and exhibited full inhibition. Behaviours that reduce competition among roots of the same individual have been reported for several other species. When in contact with self they develop fewer and shorter roots compared with the contact with non-self individuals (Gersani *et al.* 2001; Holzapfel & Alpert 2003; Falik *et al.* 2003; Gruntman & Novoplansky

2004; Dudley & File 2007; although see Hess & de Kroon 2007). In these studies, cuttings that were physically separated but genetically identical were perceived as non-self individuals. These results suggest that physiological connections are required for self-recognition by roots and do not support the involvement of recognition based on underlying genetics, typical of mammalian immunity and plant reproductive self-incompatibility.

In this study, we explored self-recognition in the context of plant resistance to herbivory. Previously, we found that sagebrush (*Artemisia tridentata*) became more resistant to herbivores after exposure to volatile cues from experimentally damaged neighbours (Karban *et al.* 2004, 2006; Shiojiri & Karban 2006). Plants within 60 cm of an experimentally clipped neighbour in the field experienced less leaf damage over the season compared with plants near an unclipped neighbour; plants with root contact between neighbours but not air contact failed to show this response. Naturally occurring herbivores caused similar responses as experimental clipping with scissors (Shiojiri and Karban 2008) and active cues were released for up to 3 days following clipping (Shiojiri *et al.*, in press). Choice and no-choice experiments indicated that herbivores responded to changes in plant characteristics and were not being repelled directly by

airborne cues released by clipped individuals (Karban and Baxter 2001).

Volatile cues were also required for systemic induced resistance among branches on an individual; vascular connections were insufficient (Karban *et al.* 2006). This last result suggested that volatile cues may allow individuals to integrate physiological processes such as systemic-induced resistance. Volatile cues have been found to allow intraplant communication leading to systemic-induced resistance in other systems as well (Frost *et al.* 2007; Heil & Silva Bueno 2007). These previous results stimulated the questions that are addressed here: (i) Do sagebrush individuals communicate more effectively with self rather than non-self branches? (ii) Is physical connection required for sagebrush to distinguish between self and non-self, or might some genetic recognition system be involved?

MATERIALS AND METHODS

We answered these questions by making cuttings of 30 sagebrush individuals and placing these potted clones near either their genetically identical 'self parent' (treatment 3 in Fig. 1) or near a non-self plant (treatment 4 in Fig. 1). While *A. tridentata* does not normally reproduce clonally we used the potted cuttings as potential emitters of cue. They were experimentally clipped early in the season. The rooted parents served as assay plants on which we measured accumulated natural herbivore damage over the season, using different plants in 2007 and 2008. Two control assays were established: an assay that was not manipulated (treatment 1 in Fig. 1) and an assay with a branch that had physical connections with an experimentally clipped branch (treatment 2 in Fig. 1). These were not true controls since they had not been propagated to produce cuttings and they lacked potted neighbours during the experiment (Fig. 1), although they were useful as references in comparisons of herbivore damage. It was expected that the assay with unclipped neighbours (treatment 1) would not induce resistance and would experience the most natural damage and that the assay attached to a clipped branch (treatment 2) would induce the maximum resistance and experience the least natural damage. If sagebrush communicates more effectively with self rather than non-self branches, it was expected that the assay with self-communication (treatment 3) would induce greater resistance and experience less natural damage than the non-self assay (treatment 4).

Eighty 'young' sagebrush plants (*sensu* Shiojiri & Karban 2006) with a canopy diameter of *c.* 30 cm were selected along the south edge of Taylor meadow (39°26.663 N, 120°14.748 W, 1932 m elevation, UC Sagehen Creek Natural Reserve) in each year and randomly assigned to one of the four treatments (Fig. 1, such that $n = 25$ for

treatments 1, 2 and $n = 15$ for treatments 3, 4). During the dormant season, cloned cuttings were made from plants in treatments 3 and 4 by crown division (Hartmann & Kester 2002). Cuttings were placed in 3.8-L plastic pots and grown outdoors in Davis, CA. Potted cuttings were returned to the field and placed either near the parent from which they had been cloned (self, treatment 3) or near another different assay plant (non-self, treatment 4) on 14 May 2007 or 22 May 2008 such that the canopy of the potted cutting was within 5 cm of the canopy of the assay branch. All assay plants of treatments 3 and 4 had cuttings taken from their root crowns during the previous winter. Treatments 3 and 4 were compared using 15 independent self or 15 non-self pairs of plants in each year. One branch of all plants in treatments 2, 3 and 4 had the distal edge of one-third of the leaves clipped with scissors on 15 May 2007 or 22 May 2008. We measured herbivory by counting the number of leaves with any visible damage caused by herbivores on assay branches of plants of all treatments on 10 September 2007 or 12 September 2008. Since assay branches varied in the number of leaves, the number of leaves with damage was standardized for branches of 100 leaves. We have used this presence/absence measure of herbivory in all of our previous work in this system and found that it correlates with the percentage of leaf area removed. Treatment effects were examined using ANOVA (JMP 7.0) of the standardized number of damaged leaves for data pooled from the two years. Specific *a priori* hypotheses were tested using planned contrasts.

RESULTS

Damage rates by naturally occurring herbivores were 26% higher in 2008 than 2007 (Fig. 2; $F_{1,152} = 4.3$, $P = 0.04$) although treatment effects were not different over the two years (interaction $F_{3,152} = 1.4$, $P = 0.25$); year was not included in subsequent models. Damage was caused primarily by generalist grasshoppers (*Cratypedes neglectus* Thomas, *Camnula pellucida* Scudder, *Trimerotropis fontana* Thomas and *Lepus intermedus* Saussure). Damage by herbivores to plants of the four treatments differed (Fig. 2, $F_{3,156} = 30.6$, $P < 0.001$). The unclipped 'control' (treatment 1) experienced more herbivore damage than the other three treatments (Fig. 2), consistent with expectations (*a priori* contrast; $F_{1,156} = 78.7$, $P < 0.001$). Of more interest, the self assay (treatment 3) accumulated 42% less herbivore damage than the non-self assay (treatment 4), consistent with expectations (*a priori* contrast; $F_{1,156} = 6.0$, $P = 0.015$). This result suggests that the self assay plants responded more effectively to the cues emitted by the clipped clones than did the non-self assay plants. The two 'control' treatments (1 and 2) had not been propagated and lacked potted neighbours that could have affected the rates

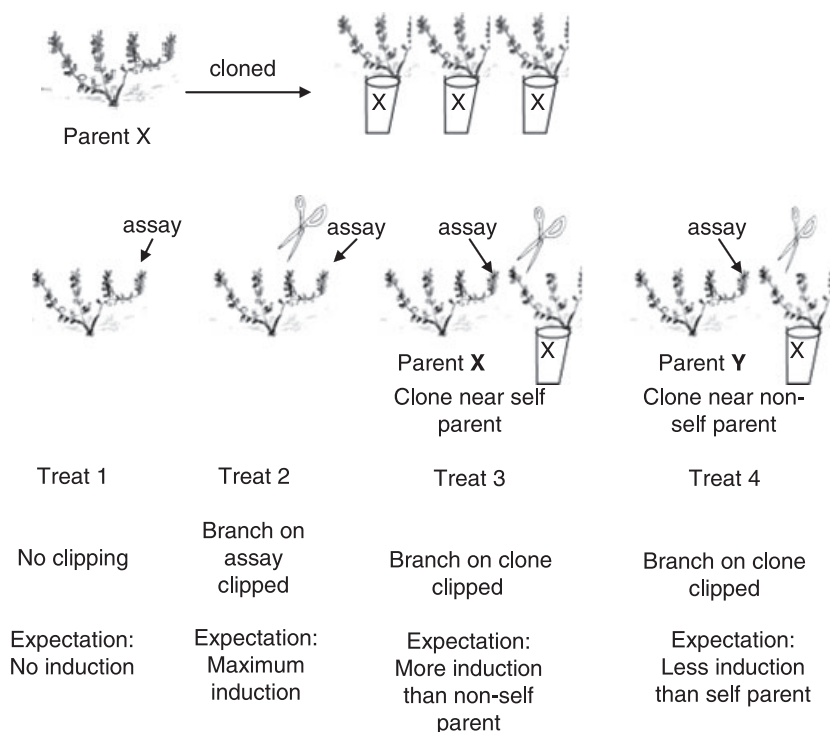


Figure 1 Design of the field experiment showing plant cloning, the four treatments and *a priori* expectations. Fifteen different plants were cloned although x and y in the figure refer to the comparison of same or different genotypes. In each year, there were 15 independent self pairs and 15 non-self pairs. The scissors indicate which branch received experimental clipping at the start of the season and the assay refers to the branch that was evaluated for herbivore damage at the end of the season.

of herbivory. However, our primary goal was to compare self and non-self communication and these treatments (3 and 4) experienced similar conditions throughout the experiment and differed only in the identity of their clipped neighbour.

DISCUSSION

This is the first demonstration of self, non-self recognition involving plant communication and defense. Unlike other examples of plant recognition that required direct physiological connection between ramets (references given before), sagebrush in this study responded differently to cues of self and non-self in the absence of physical contact. Known systems of discrimination that rely on recognition of different allotypes require direct contact of highly specific cell-surface determinants (Grosberg 1988; Dixit & Nasrallah 2001). The mechanisms of recognition involved in this interaction are not known although volatile rather than surface determinants are likely. We examined the relationship between the volatile profiles of clipped plants and herbivore damage of assays and this examination revealed only high levels of variation among individuals (see Table S1 in Supporting Information). High variability among signals has been predicted to be adaptive in recognition systems (Wright 1939) and has been found in self-incompatibility systems in plants (e.g. Takayama *et al.* 2000; Dixit and Nasrallah 2001; Edlund *et al.* 2004) and self-recognition among clonal invertebrates (Grosberg 1988). The observa-

tion that individuals possess high variability of volatiles emitted following clipping is consistent with the hypothesis that volatiles serve as cues, although other factors could also produce this variability.

Volatile cues are required for communication among branches within an individual sagebrush plant (Karbon *et al.* 2006). This observation suggests that communication between individuals may be a by-product of a volatile communication system that allows plants to integrate their own systemic physiological processes (Karbon *et al.* 2006; Frost *et al.* 2007; Heil & Silva Bueno 2007). Our findings here that volatiles are variable among individuals and have some individual specificity are consistent with this hypothesis that they function primarily in self-communication among different parts of the genetic individuals. One potential cost of a volatile communication system is eavesdropping by non-self neighbours (Bruin & Dicke 2001; Heil & Ton 2008); Why should individuals emit cues that are beneficial for neighbours with whom they compete?

Recent findings indicate that volatile cues are also used by autotrophic plants to recognize and orient away from competitors (Pierik *et al.* 2003, 2004) and by parasitic plants to recognize and orient towards suitable hosts (Runyon *et al.* 2006). The current study indicates that plants respond differently to volatile cues from self and non-self ramets that have been experimentally clipped. The ability to recognize relatedness is a necessary prerequisite for kin selection, and self, non-self discrimination is the most rudimentary step in that direction (Callaway & Mahall 2007).

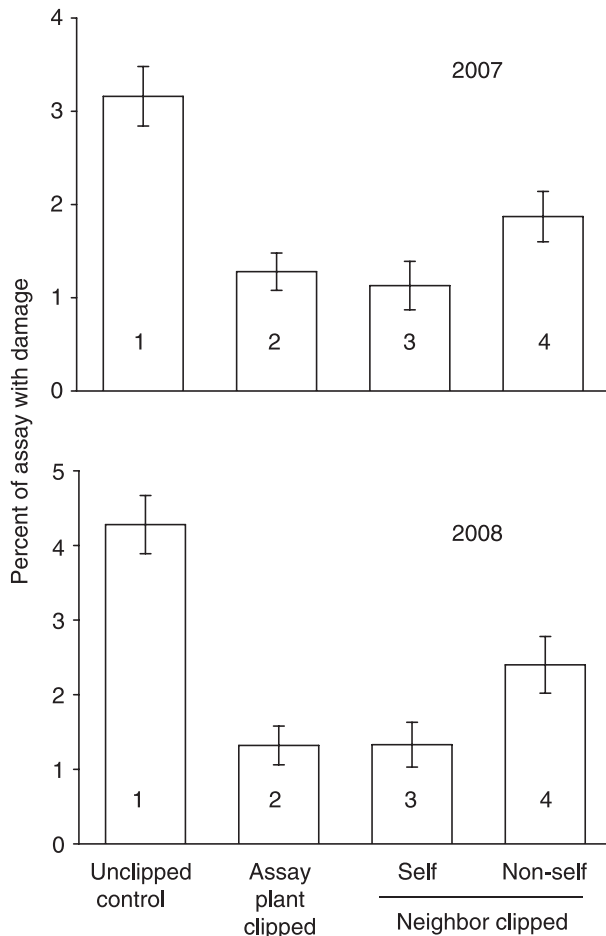


Figure 2 Mean (± 1 SE) levels of natural herbivore damage standardized for assay branches of 100 leaves on plants of the four treatments in 2007 and 2008. The numbers inside each histogram refer to the treatments illustrated in Fig. 1.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Variable volatile profiles from sagebrush individuals.

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