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[SHORT COMMUNICATION]

Reconsidering the avoidance of spider mite *Tetranychus urticae* Koch webs by bush killer tendrils *Cayratia japonica* (Thunb.) Gagnep.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) feeds on hundreds of agricultural crops across many plant families (Jeppson et al., 1975). Plants have developed various ways to reduce feeding damage caused by spider mites, such as direct chemical defenses (Li et al., 2002), and indirect defense providing alternative foods for predatory mites (Ozawa and Yano, 2009).

Cayratia japonica (Thunb.) Gagnep., 1911 (Vitales: Vitaceae) is a common perennial vine that reproduces via seeds and rhizomes. *Cayratia japonica* readily regenerates from rhizome fragments (West et al., 2012), coils around neighboring plants using tendrils, and typically smothers these support plants once established (Brown, 1992). Therefore, it is generally regarded as an exceptionally undesirable species (e.g. West et al., 2011). The tendrils of *C. japonica* show a sophisticated behavior in avoiding coiling around conspecifics, a product of contact chemoreception (Fukano, 2017). It is presumed that the fitness of *C. japonica* individuals is reduced when they coil around themselves or closely related individuals, i.e., a reduction in inclusive fitness. Furthermore, *C. japonica* plants coiling around support plants infested with ambulatory spider mites will be infested by mites entering from the support plants. Generalist predatory mites that also feed on pearl bodies on the surface of *C. japonica* (Ozawa and Yano, 2009) may control a small number of spider mites on the plant, however, mass inflow of mites should cause severe damage to the plant. The tendrils reportedly also avoid coiling on support plants that are covered in the webs of spider mites (Nakai and Yano, 2019), which should function as an anti-herbivore defense. *Cayratia japonica* distributes not only in natural ecosystems but also in agricultural ecosystems

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つる草ヤブガラシによるハダニ網への巻きつき回避反応を再考する
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(Kitamura and Murata, 1961) where *T. urticae* density is high. Therefore, *C. japonica* plants are likely to encounter support plants heavily infested by *T. urticae* in such environments.

Broadly, anti-herbivore defenses adopted by plants depend on resource availability in their environment (Coley et al., 1985) as well as life stage (Sobral et al., 2021). Although the rhizomes of wild *C. japonica* can spread longer than 2 m, studies concerned with coiling behavior have exclusively used small potted specimens grown from rhizome fragments. The coiling behaviors of wild *C. japonica* plants with full rhizomes, as well as under different light intensities, remain unexplored. We examined the coiling behavior of wild and potted *C. japonica* plants to address this knowledge gap.

MATERIALS AND METHODS

Mites

The polyphagous spider mite *T. urticae* infests hundreds of host plant species across many plant families (Jeppson et al., 1975), including *C. japonica*. This species is therefore highly likely to infest the support plants of *C. japonica*. We obtained a population of *T. urticae* from *Chrysanthemum morifolium* Ramat., 1792 (Asterales: Asteraceae) in Nara, Japan, in 1998. Mites were reared on potted kidney bean *Phaseolus vulgaris* L., 1753 (Fabales: Fabaceae), which is a preferred host (Yano et al., 1998), maintained at 25°C and 50% relative humidity. The webs of *T. urticae* were collected from *P. vulgaris* and used in the experiments detailed below.

Plants

Wild *C. japonica* was identified and collected from the campus grounds of Kyoto University in April–May 2021. All collected specimens were growing naturally under direct light. During May–June 2021, we collected *C. japonica* individuals, including their root fragments (average length, 20 cm), and transplanted them to plastic pots (12 cm diameter, 10 cm depth) filled with commercial soil (Engeibaiyoudo; Tachikawa Heiwa Nouen, Kanuma, Japan). Transplants were maintained in a well-ventilated greenhouse under near-natural light intensity at Kyoto University for one month prior to experimentation. Main stems were supported using wire when necessary to allow for the free movement of tendrils.

Coiling responses of wild **C. japonica** *to spider mite webs*

To create target objects covered with spider mite webs, we created triangular pyramid-shaped structures out of filter paper (length, 55 mm) and covered the top 30 mm of each pyramid with *T. urticae* webs by slashing the webs three times by the pyramid (Fig. 1a). The webs contained feces, exuviae, and live mites in all developmental stages. Naked filter paper pyramids were used as controls.

Before exposing wild *C. japonica* to the pyramids, we observed their tendrils for 30 minutes to forecast their movement. We then set up a control (n = 44) or web-covered (n = 41) pyramid in the projected direction of travel using wire and flexible poles (Yoransen, 1,000 mm; Watanabetai Co., Ltd., Osaka, Japan) (Fig. 1b). We observed each tendril 5 hours after they had touched a pyramid. Tendrils were considered to have coiled when they had rotated around the filter paper

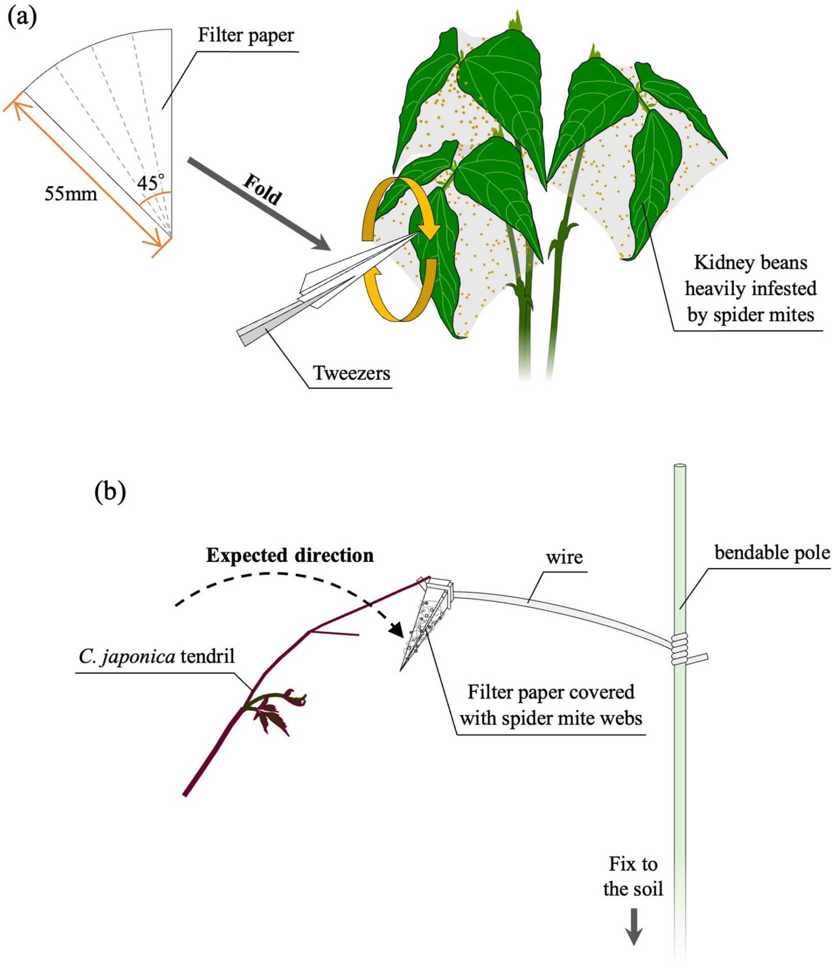

Fig. 1. (a) The preparation of a filter paper pyramid covered with spider mite webs. (b) Experimental design used to investigate the coiling behavior of *C. japonica* tendrils to objects covered with spider mite webs.

piece > 180°. We observed each *C. japonica* individual only once to preclude any possible effect from a previous trial. All observations described above were conducted between May and June in 2021. We then compared the proportions of tendrils coiled around pyramids with and without spider mite webs using Fisher's exact test.

Coiling responses of potted **C. japonica** *to* **T. urticae** *webs in a greenhouse*

Potted *C. japonica* individuals ca. 30 days after transplantation were used. Potted individuals had lower aboveground biomass than their wild counterparts, as estimated visually. Specimens selected for experimentation were > 20 cm in height and bore active tendrils. We observed the tendrils for 15 minutes before experimentation to forecast their movement and then affixed web-covered (n = 15) or control (n = 14) pyramids in their projected direction of travel using wire and bendable poles (Yoransen, 600 mm; Watanabetai Co., Ltd., Osaka, Japan). Tendril movement was then observed in the same manner as described for the wild plants, and each potted specimen was only used in a single trial. Fisher's exact test was used to compare the proportion of tendrils coiled around the control and web-covered pyramids.

RESULTS

Coiling behavior of wild **C. japonica**

The proportions of wild *C. japonica* tendrils coiled around control and web-covered pyramids (i.e. rotated around the filter paper pieces > 180°) did not differ significantly (P = 1.0000; Fig. 2a), indicating that wild *C. japonica* plants did not avoid spider mite webs.

Coiling behavior of potted **C. japonica**

Significantly fewer potted *C. japonica* tendrils coiled around the web-covered pyramids relative to the control pyramids (P = 0.0025; Fig. 2b), indicating that potted *C. japonica* tendrils avoided spider mite webs under near-natural light intensity.

DISCUSSION

Wild *C. japonica* plants did not avoid coiling in the presence of *T. urticae* webs. This finding contradicts that of Nakai and Yano (2019), who reported the avoidance of *T. urticae* webs by potted *C. japonica* plants. The wild specimens used here and the potted specimens used by Nakai

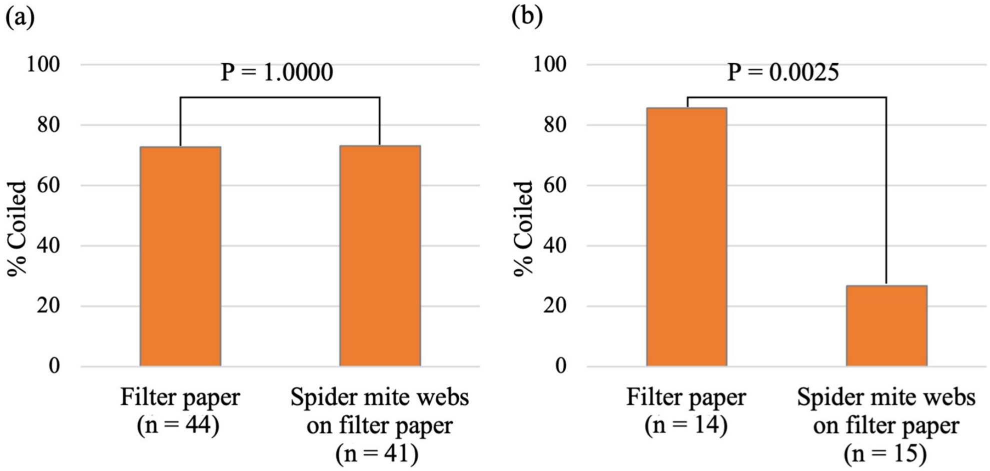

Fig. 2. Coiling responses of (a) wild and (b) potted *C. japonica* tendrils to filter paper pyramids covered with spider mite webs.

and Yano (2019) differed in some regard. First, the belowground biomass of potted *C. japonica* reared in 500-mL pots (started from 20 cm root fragments) would likely be much lower than that of wild *C. japonica*, which can extend longer than 2 m via the rhizomes (Fukano and Yamawo, 2015). Second, the rearing and experimental conditions of Nakai and Yano (2019) included a light intensity of 3,000 lux, which is approximately one tenth of natural light intensity. Therefore, this discrepancy in experimental results may be due to differences in plant biomass or light intensity.

Our second experiment aimed to address the role of light intensity in the coiling behavior of potted *C. japonica* and thus elucidate if this factor may have contributed to our novel finding. We found that potted plants grown under near-natural light intensity showed the same response to webs of *T. urticae* reported by Nakai and Yano (2019). Thus, we can reasonably suggest that coiling behavior in *C. japonica*, as it pertains to the webs of *T. urticae*, is dependent on biomass, rather than environmental light conditions. We suspect this pattern may be because small individuals would suffer a great deal of damage due to spider mite infestation upon coiling around an infested support plant, but this same level of infestation may be negligible to a large individual. Although spider mites are capable of reproducing on *C. japonica* plants, mites that flow into larger plants are more likely to be controlled by generalist predators that also feed on pearl bodies on the plants (e.g. Ozawa and Yano, 2009). Thus, the cost of potential feeding damage to large individuals may be outweighed by the cost of avoiding an available support plant. Nakai and Yano (2019) suggested that it may be possible to manufacture fencing materials that would be avoided by *C. japonica*, if the proximate factors underlying the coiling avoidance of spider mite webs were known. We suggest that this effort would be in vain given the lack of avoidance shown by wild *C. japonica*.

Finally, our results have prompted questions regarding the selective coiling behavior demonstrated by *C. japonica* to their own stems or those of related conspecifics (Fukano, 2017; Fukano and Yamawo, 2015). Studies demonstrating this behavior exclusively used potted plants grown from rhizome fragments. Thus, this behavior is only representative of plants with low biomass and should be more carefully assessed when projecting the spread of wild *C. japonica*. Further work to clarify if this behavior remains constant across plants of increasing biomass is encouraged.

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