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LEGUMINOUS COVER CROPS AND THEIR INTERACTIONS WITH CITRUS AND DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Three legume species with potential as cover crops in citrus groves were studied for their effect on the developmental biology of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) in greenhouse studies. All 3 cover crops were hosts for the Diaprepes root weevil, *Cajanus cajan* (pigeon pea) was a superior host for development of *D. abbreviatus* compared with citrus rootstocks. *C. cajan* appeared to be allelopathic; the root mass of uninfested citrus was greatly reduced when grown in association with *C. cajan* compared with citrus grown alone. Association of citrus with *C. cajan* or *Arachis pintoi* (perennial peanut) reduced chlorophyll fluorescence, a measure of photosynthesis, compared with citrus associated with *Crotalaria pallida* (rattlebox) or with another citrus seedling. When grown in close association with *A. pintoi*, citrus produced the same amount of root mass as citrus seedlings grown alone. Infestation with larval *D. abbreviatus* reduced chlorophyll fluorescence of citrus by 26%. None of the 3 legume species tested reduced the feeding damage caused by *D. abbreviatus* to citrus. Larvae reared in pots with *A. pintoi*, associated with citrus or alone, gained weight at the same rate as larvae reared on the citrus rootstocks alone. Larvae recovered from pots containing *C. pallida* associated with citrus weighed significantly more than larvae reared on citrus alone. *C. cajan* appears to be particularly inappropriate as a cover crop because of its positive effect on larval growth and reduction of citrus root mass. None of the 3 legume species tested had a negative effect on *D. abbreviatus* or on feeding damage.

Key Words: Citrus, *Diaprepes abbreviatus*, cover crops, perennial peanut, *Arachis pintoi*, pigeon pea, *Cajanus cajan*, *Crotalaria pallida*

RESUMEN

Tres especies de leguminosas forrajeras con potencial como cobertura en cítricos fueron evaluadas por su efecto sobre el desarrollo del cucarrón *Diaprepes abbreviatus* (L.) en un invernadero. Las tres especies sirvieron como hospedantes para el insecto. *Cajanus cajan* (gandul) fue un hospedante superior para el desarrollo de *D. abbreviatus* comparado con patrones de cítricos. *C. cajan* fue alelopático; la masa de raíces de cítricos no-infestadas fue menor en asociación con *C. cajan* comparado con cítricos solos. La asociación de cítricos con *C. cajan* o *Arachis pintoi* (mani forrajero) resultó en una reducción de fluorescencia de clorofilia, una medida de fotosíntesis, comparado con cítricos asociados con *Crotalaria pallida* o con otra planta de cítricos. Cítricos asociados con *A. pintoi* produjeron la misma cantidad de raíces que los cítricos solos. La infestación con larvas de *D. abbreviatus* resultó en una reducción de 26% en fluorescencia de clorofila de plántulas de cítricos. Ninguna de las tres especies de leguminosas redujo el daño causado por alimentación de *D. abbreviatus* en cítricos. Las larvas en potes con *A. pintoi* (asociado con cítricos o solo) aumentaron de peso de la misma manera que larvas sobre raíces de cítricos solo. Larvas recuperadas de potes que contenían *C. pallida* asociado con cítricos pesaron mas que larvas sobre cítricos solo. Parece que *C. cajan* en particular no es recomendable como cobertura debida a su efecto positivo sobre el crecimiento de larvas y su efecto negativo sobre la masa radicular de cítricos. Ninguna de las tres leguminosas tuvo un efecto negativo sobre *D. abbreviatus* o redujo su daño. Translation provided by author.

Leguminous cover crops can contribute to increased and sustainable crop productivity through erosion and weed control, biological nitrogen fixation, and by providing refuge for natural enemies of arthropod pests (Hokkanen 1991). Cover crops such as perennial peanut (*Arachis* spp.) have been suggested for use in citrus groves (Prine et al. 1981), but adoption of this practice will depend on an unequivocal demonstration of benefits to grove managers. Cover crops should be selected, at a minimum, that do not harbor key pests and may be selected to divert or deter pests and contribute to the diversity and abundance of natural enemies (Altieri 1995, Risch 1981).

A major concern to Florida citrus producers is the highly polyphagous *Diaprepes* root weevil, *Diaprepes abbreviatus* (L.) (Simpson et al. 1996). Cover crops or trap crops could contribute to citrus productivity and control of damage from *D. abbreviatus*. It is equally possible, however, that
introduction into the citrus cropping system of an additional plant resource for a highly polyphagous pest could result in higher pest population density (Andow 1991), particularly in the case of *D. abbreviatus* in Florida where natural enemies are insignificant (Hall et al. 2001).

As a first attempt to study the potential influence of cover legumes on the biology of *D. abbreviatus*, I examined the response of larvae to 3 legume species in a greenhouse. *Arachis pintoi* Krapovickas & Gregory is increasingly used as a tropical forage and as a cover in diverse tropical tree crops (de la Cruz et al. 1993) and has been considered for use in Florida citrus groves. *Cajanus cajan* Millspaugh (pigeon pea) is widely grown in Puerto Rico where anecdotal observations suggest a strong preference for this species by *D. abbreviatus*. *Crotalaria pallida* Ait. (rattlebox) has been used extensively in Florida as a green manure and has become naturalized. I report here the effect of these species alone and associated with citrus on development of *D. abbreviatus*.

**MATERIALS AND METHODS**

**Trial I. Effect of Plant Associations in 3.8-L-pots.**

Seed of *C. cajan*, *A. pintoi* and *C. pallida* were planted in germination trays in a soilless potting mix (Metromix 500, Scotts, Marysville, OH) and transplanted at approximately 1 mo after germination. Seedlings of 'Carrizo' citrange (*C. sinensis* (L.) Osbeck × *P. trifoliata*) were germinated in fine, sterile sand (Bonsal Play Sand, W. R. Bonsal Co., Charlotte, NC) and transplanted to 3.8-L-pots at 4 mo after germination. All plants were transplanted to 3.8-L-pots containing sterile sand. The pots were lined with a nylon mesh cloth to prevent escape of larvae through the drainage holes. Pots were planted with 2 seedlings per pot in the following combinations: two seedlings of either 'Carrizo', *A. pintoi*, *C. cajan*, or *C. pallida*; or one seedling of 'Carrizo' and a companion plant of *A. pintoi*, *C. cajan*, or *C. pallida*. An additional combination consisted of one plant of *C. cajan* and one plant of *A. pintoi*. Treatments consisted of 3 weevil-infested and 3 noninfested pots of each of 8 plant combinations in a randomized block design. A total of 48 pots were arranged randomly on greenhouse benches within infested and noninfested blocks. Ten early instar larvae of *D. abbreviatus* weighing 20 ± 5 mg each were added to infested pots on 30 June 1998. Larvae were obtained from a laboratory colony maintained by the U.S. Horticultural Research Laboratory, Orlando, FL and reared according to Lapointe & Shapiro (1999). Early instars were used instead of neotenates to avoid escape or movement of larvae between pots. Citrus seedlings were one year old and legume seedlings were 3 mo. old at the beginning of the infestation period. Plants were maintained throughout the experiment on elevated benches in a greenhouse with an average diurnal temperature cycle of 35°C maximum and 23°C minimum. No supplemental light was supplied. Maximum photosynthetic photon flux in the greenhouse was 800 mol·s⁻¹·m⁻². Plants were watered with a dilute fertilizer mix weekly using water-soluble 20N-10P-20K at a rate of 150 mg·liter⁻¹·N.

At the end of the infestation period, an OSL-FL modulated chlorophyll fluorometer (Opti-Sciences, Tyngsboro, MA, USA), was used to measure yield of chlorophyll fluorescence of light-adapted leaves with saturation intensity of ~2.7 kue² for 0.8 sec. Yield (relative units) is an indicator of quantum yield of photosynthesis and is often used in measuring plant stress (Schreiber & Bilger 1987, van Kooten & Snel 1990). Chlorophyll fluorescence was measured during the late morning hours (9:00-11:00). All readings (Y values) were taken from the center of a leaf, and always from the center leaflet in the case of trifoliate leaves. Readings were taken from 3 positions on each plant: 'top' was taken from the first fully expanded leaf, 'bottom' was taken from the lowest available intact leaf, and 'middle' was taken from the leaf at the mid-point between 'top' and 'bottom'.

The plants and sand were removed from the pots and sieved to recover larvae 37 d after infestation on 6 August 1998. Recovered larvae were counted and weighed before and after drying in an analytical oven at 60°C for ≥48 h to obtain fresh and dry weights. Roots were washed and separated from the above-ground portion by cutting at the point where the first (uppermost) lateral root emerged from the central root. Roots were allowed to air-dry and weighed.

The effect of plant combination on total larval weight per pot, and the effect of infestation and plant combination on citrus root weight and chlorophyll fluorescence were compared by ANOVA. Where appropriate, means were compared by Tukey's Honestly Significant Differences (HSD) test (Abacus Concepts 1996).

**Trial II. Effect of Plant Associations in 76-L-pots.**

Eighteen 1-year-old seedlings of the rootstock 'Sun Chu Sha' mandarin (*C. reticulata* Blanco) were transplanted to 76-L-pots containing sterile sand in May, 1998. Availability of plants determined choice of rootstock for this trial. However, 'Carrizo' and 'Sun Chu Sha' have been shown to be equivalent in terms of larval weight gain of *D. abbreviatus* reared on potted seedlings, and in terms of damage to roots of seedlings infested with *D. abbreviatus* (Lapointe et al. 1999).

Plant combinations in the pots consisted of either a single tree of 'Sun Chu Sha' or a seedling of 'Sun Chu Sha' surrounded by 10 seedlings of...
**RESULTS**

Trial I. Effect of Plant Associations in 3.8-L-pots.

Both companion plant and state of infestation had a significant effect on chlorophyll fluorescence of ‘Carrizo’ leaves. There was no significant effect from leaf position ($F = 2.4; df = 2, 72; P = 0.10$), interaction between infestation and companion plant ($F = 1.8; df = 3, 72; P = 0.16$) or between infestation and position ($F = 0.5; df = 2, 72; P = 0.59$). For analysis of effects of infestation and companion plant, the measures of chlorophyll fluorescence for top, middle, and bottom ‘Carrizo’ leaves were pooled.

Infestation with larval *D. abbreviatus* reduced chlorophyll fluorescence of the ‘Carrizo’ plants by $26 \pm 8\%$ compared with noninfested controls ($F = 23.7; df = 1, 72; P < 0.01$). The species of companion plant also significantly affected chlorophyll fluorescence of ‘Carrizo’ ($F = 3.3; df = 3, 72; P = 0.03$). ‘Carrizo’ planted with another ‘Carrizo’ plant or planted with *C. pallida* had higher levels of chlorophyll fluorescence compared with ‘Carrizo’ plants planted with either *A. pintoi* or *C. cajan*. Association with *A. pintoi* or *C. cajan* reduced photosynthesis fluorescence in ‘Carrizo’ by 20% (Table 1).

There was neither an effect of companion ($F = 0.9; df = 2, 66; P = 0.40$) nor of infestation ($F = 0.1; df = 1, 66; P = 0.73$) on the chlorophyll fluorescence of *C. cajan*. There was an effect of leaf position on chlorophyll fluorescence ($F = 9.1; df = 2, 66; P < 0.01$). Top and middle *C. cajan* leaves had higher levels of fluorescence ($0.514 \pm 0.015$ and $0.482 \pm 0.024$, respectively) than lower leaves ($0.389 \pm 0.024$) (Tukey’s HSD, $\alpha = 0.05$).

### Table 1. Yield of Chlorophyll Fluorescence (Mean Relative Value ± SE) of a Citrus Rootstock (‘Carrizo’) Planted in 3.8-L-Pots with 3 Species of Legume Companion Plants.

| Companion       | Yield         | n  |
|-----------------|---------------|----|
| *A. pintoi*     | 0.302 ± 0.028 | a  |
| *C. cajan*      | 0.308 ± 0.032 | a  |
| Citrus          | 0.386 ± 0.020 | b  |
| *C. pallida*    | 0.392 ± 0.045 | b  |

Means followed by the same letter are not significantly different at $P = 0.05$ by Tukey’s HSD after a significant ANOVA ($F = 3.3; df = 3, 72; P = 0.03$).

The chlorophyll fluorescence of *A. pintoi* was unaffected by companion ($F = 2.3; df = 2, 66; P = 0.11$) and infestation ($F = 0.9; df = 1, 66; P = 0.35$). As in the case of *C. cajan*, there was a significant effect of leaf position ($F = 4.5; df = 2, 66; P = 0.01$); top leaves had a higher rate ($0.433 \pm 0.019$) compared with middle and lower leaves ($0.344 \pm 0.024$ and $0.350 \pm 0.028$, respectively) (Tukey’s HSD, $\alpha = 0.05$).

There was no effect of companion (citrus or another *C. pallida*) ($F = 0.54; df = 1, 49; P = 0.47$) nor of infestation ($F = 0.004; df = 1, 49; P = 0.95$) on the chlorophyll fluorescence of *C. pallida*. There was a significant effect of leaf position ($F = 4.2; df = 2, 49; P = 0.02$); top leaves had a higher rate ($0.465 \pm 0.027$) than lower leaves ($0.332 \pm 0.038$) and middle leaves were intermediate ($0.372 \pm 0.032$), not significantly different from either top or lower leaves (Tukey’s HSD, $\alpha = 0.05$).

Both companion plant ($F = 7.1; df = 3, 22; P < 0.01$) and infestation ($F = 66.9; df = 1, 22; P < 0.01$) significantly affected final root weight of ‘Carrizo’ plants in 3.8-L-pots, and there was a significant interaction between companion plant and infestation ($F = 3.9; df = 3, 22; P = 0.02$). For this reason, root weights were analyzed separately for infested and noninfested groups (Table 2). Noninfested ‘Carrizo’ plants in 3.8-L-pots had a smaller root mass when associated with *C. cajan*, but not when associated with *A. pintoi* or *C. pallida*, compared with ‘Carrizo’ plants associated with another ‘Carrizo’. ‘Carrizo’ root weight was greater when grown with *A. pintoi* than with *C. pallida* or *C. cajan* (Table 2).

The root mass of all ‘Carrizo’ plants infested with *D. abbreviatus* was significantly reduced. In the case of ‘Carrizo’ associated with ‘Carrizo’, pots contained 2 plants and therefore twice as much citrus root mass was available compared with ‘Carrizo’ associated with a legume species. Apparently, weevils fed equally on ‘Carrizo’ regardless of companion plant, i.e., none of the associations resulted in reduced feeding damage to ‘Carrizo’ (Table 2). If the interaction term is ignored by setting a at 1%, the main effect of infestation by *D. abbreviatus* reduced ‘Carrizo’ root weight by 68 ± 7%.
Lapointe: Cover crops, citrus and Diaprepes abbreviatus

compared with noninfested ‘Carrizo’. Similarly, the main effect of companion plant was highly significant ($F = 7.1; df = 3, 22; P < 0.01$). When *C. cajan* or *C. pallida* were the companion plants, root weight of ‘Carrizo’ was reduced by 53 and 41%, respectively. However, when associated with *A. pintoi*, root weight of ‘Carrizo’ was equal to the root weight of individual ‘Carrizo’ trees planted with a second ‘Carrizo’ ($\alpha = 0.05$, Tukey’s HSD).

There was a significant effect of plant association on total wet weight of larvae recovered from each pot ($F = 35.4; df = 7, 15; P < 0.01$), total dry weight of recovered larvae ($F = 59.7; df = 7, 15; P < 0.01$) and on the number of larvae recovered per pot ($F = 8.3; df = 7, 16; P < 0.01$). In the 3 associations that included *C. cajan*, more ($F = 46.9; df = 1; P < 0.01$) and larger (wet weight: $F = 242.0; df = 1; P < 0.01$) larvae were recovered compared with pots containing 2 ‘Carrizo’ plants (Table 3). The other 2 legume species did not affect the number or size of larvae recovered compared with pots containing 2 ‘Carrizo’ plants.

**Trial II. Effect of Plant Associations in 76-L-pots.**

There was a significant effect of association ($F = 9.1; df = 2, 12; P < 0.01$) and of infestation ($F = 11.1; df = 1, 12; P < 0.01$) on weight of citrus roots. There was also a significant interaction between these effects ($F = 4.3; df = 2, 12; P = 0.04$). Citrus root weight differences between noninfested and weevil-infested trees differed between citrus alone and citrus with *A. pintoi* compared with citrus associated with *C. cajan*. There was no significant difference in citrus root weights between infested or noninfested citrus in association with *C. cajan*, whereas citrus root weights of noninfested citrus alone or in association with *A. pintoi* were increased by 47-55% over infested citrus (Fig. 1).

The effect of infestation was therefore tested for each treatment (association) separately, and treatment means were compared within infested and noninfested groups. Uninfested ‘Sun Chu Sha’ plants associated with *C. cajan* had much smaller root mass compared with ‘Sun Chu Sha’ alone or ‘Sun Chu Sha’ associated with *A. pintoi* (Fig. 1). Root mass of ‘Sun Chu Sha’ was equivalent for all 3 infested treatments (‘Sun Chu Sha’ alone, ‘Sun Chu Sha’/*A. pintoi*, and ‘Sun Chu Sha’/*C. cajan*).

The effect of plant association on final larval fresh weight of 15 larvae recovered from each pot was significant at $P = 0.06$ (ANOVA). However,

### Table 2. Mean Fresh Weight (±SE) of Roots of Citrus Seedlings Infested with Larval *Diaprepes abbreviatus* and Grown in 3.8-L-Pots with 3 Species of Companion Legumes or with a Second citrus Seedling.

| Companion       | Infested | Noninfested | n |
|-----------------|----------|-------------|---|
| *C. pallida*    | 0.23 ± 0.03 a | 1.75 ± 0.38 ab | 3 |
| *C. cajan*      | 0.39 ± 0.17 ab | 1.19 ± 0.10 a | 3 |
| *A. pintoi*     | 0.49 ± 0.19 ab | 2.99 ± 0.47 c | 3 |
| Citrus          | 1.12 ± 0.21 b | 2.21 ± 0.18 bc | 6 |

Means in a column followed by the same letter are not significantly different at $P = 0.05$ by Tukey’s HSD after a significant ANOVA.

### Table 3. Mean Number and Total Weight (±SE, n = 3) of Larval *Diaprepes abbreviatus* Recovered from 3.8-L-Pots Containing 8 Different Plant Associations.

| Association       | No. larvae recovered | Wet | Dry |
|-------------------|----------------------|-----|-----|
| Citrus/Citrus      | 1.7 ± 0.3 a          | 70.4 ± 23.4 a | 16.2 ± 7.7 a |
| *C. pallida*/C. pallida | 1.7 ± 0.9 a    | 87.8 ± 23.4 a | 13.6 ± 4.2 b |
| *C. pallida*       | 1.7 ± 0.3 a          | 161.1 ± 24.0 a | 38.8 ± 8.2 b |
| *A. pintoi*/A. pintoi | 3.3 ± 0.9 ab    | 166.5 ± 47.0 a | 30.0 ± 9.4 a |
| Citrus/A. pintoi   | 3.3 ± 0.3 ab         | 210.7 ± 7.1 a  | 40.1 ± 0.6 a  |
| Citrus/*C. cajan*  | 5.0 ± 0.6 b          | 852.8 ± 53.7 b | 280.8 ± 32.4 b |
| C. cajan/C. cajan  | 5.0 ± 0.6 b          | 966.9 ± 145.8 b | 311.2 ± 42.5 bc |
| C. cajan/A. pintoi | 5.7 ± 0.3 b          | 1027.0 ± 95.0 b | 399.4 ± 7.3 c |

Means in a column followed by the same letter are not significantly different at $P = 0.05$ by Tukey’s HSD after a significant ANOVA.
the effect of plant association on final larval dry weight of 15 larvae was highly significant ($F = 23.5; \text{df} = 2, 6; P < 0.01$). The dry weight of larvae recovered from pots containing ‘Carrizo’/\textit{C. cajan} was 38% greater than that of larvae from the other 2 treatments (Table 4).

**DISCUSSION**

Damage by subterranean larvae is difficult to detect and plant damage often is not evident until days or weeks after feeding occurs. In this trial, we were unable to visually detect differences between infested and noninfested plants over the period of infestation (1 mo). Indeed, it is not uncommon in these tests to find seedlings with extensive root damage without any visual foliar symptoms. However, we were able to detect a significant reduction in chlorophyll fluorescence due to larval feeding using a fluorometer. This method may be useful in citrus groves as an indicator of tree health in general and presence of root weevils in particular.

Ground covers offer advantages and disadvantages when incorporated into agricultural production systems. In recent years, the tropical forage \textit{A. pintoi} has shown potential for use as a cover crop in tropical tree crops such as coffee, banana, oil palm, macadamia, and heart-of-palm (de la Cruz et al. 1993) and has been proposed for use in Florida’s subtropical citrus groves (Prine et al. 1981). While slow to establish, \textit{A. pintoi} is effective at weed suppression, has a non-twining growth habit, and is efficient at fixation of atmospheric nitrogen (Thomas 1993). For citrus, these attributes must be considered in relation to the potential for nutrient competition, ease of management, and effect on pests and diseases. This study indicates that \textit{A. pintoi} is the most appropriate of the 3 species studied here as a cover crop in citrus in terms of its effect on a major pest, \textit{D. abbreviatus}.

\textit{C. pallida} did not decrease chlorophyll fluorescence or root growth by the citrus rootstock. The larvae recovered from pots containing \textit{C. pallida} associated with rootstock, however, weighed significantly more than larvae reared on rootstock alone. This, combined with the upright, annual growth habit of \textit{C. pallida} make this legume a less desirable option as a cover crop compared with \textit{A. pintoi}.

\textit{C. cajan} (pigeon pea) has been reported to be attractive to \textit{D. abbreviatus} (Barrow 1924). In the tests reported here, \textit{C. cajan} was a superior host for development of \textit{D. abbreviatus} compared with ‘Carrizo’. More and larger larvae survived in pots when \textit{C. cajan} was present, regardless of association with another plant species (Tables 3 and 4). In addition, the root mass of noninfested rootstock seedlings was greatly reduced when grown in as-

| Association       | Initial | Final wet | Final dry |
|-------------------|---------|-----------|-----------|
| Citrus/\textit{A. pintoi} | 73.1 ± 2.6 a | 2480.3 ± 185.3 a | 714.3 ± 60.4 a |
| Citrus/Sun Chu Sha | 71.1 ± 2.8 a | 2604.4 ± 218.1 a | 767.3 ± 66.2 a |
| Citrus/\textit{C. cajan} | 70.9 ± 5.1 a | 3207.3 ± 128.8 a | 1191.3 ± 27.3 b |

*Means in a column followed by the same letter are not significantly different at $\alpha = 0.05$ by Tukey’s HSD following a significant ANOVA ($F = 23.5; \text{df} = 2, 6; P < 0.01$).
association with *C. cajan* compared with rootstock grown alone or in association with *A. pintoi* (Table 2, Fig. 1). The combination of increased growth of Diaprepes root weevil and apparent allelopathic effects on citrus makes *C. cajan* a particularly inappropriate choice for a cover crop.

When *A. pintoi* was grown in close association with a citrus rootstock in 3.8-L pots, the rootstock produced the same amount of root mass as citrus plants grown alone (Table 2, Fig. 1). Although none of the 3 legume species tested reduced the feeding damage caused by *D. abbreviatus* to the rootstock, larvae reared in associations that included *A. pintoi* gained weight at the same rate as larvae reared on citrus alone (Table 3). Similarly, larvae reared in pots with *A. pintoi* alone gained the same amount of weight as larvae reared on citrus alone (Table 3). Simpson et al. (1996) reported larvae feeding on the roots of peanut (*Arachis hypogaea*) and the results presented here indicate that *A. pintoi* is also a host of *D. abbreviatus*. This presents the danger of increased pest populations in citrus/*A. pintoi* polycultures, but also the possibility of diversion of larval infestation from the principal crop (citrus) to the cover crop. Andow (1991) surveyed published reports of the effect of crop diversity on pest density and found that a minority (15%) of species were more abundant in polycultures while 52% were less abundant compared with monocultures. Attempts to establish cover crops in citrus should monitor key pests such as *D. abbreviatus*. Although none of the species tested here had negative effects on *D. abbreviatus* larvae, legumes are known to be a rich source of phytochemicals with diverse insect antifeedant and toxic properties (Simmonds et al. 1990) and should be surveyed for their activity against *D. abbreviatus* for possible inclusion in citrus production systems.

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