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## **Ecological and Biological Insights into *Cuscuta planiflora*: A Fundamental study exploring its Morphology and Parasitic Behavior on *Rhanterium epapposum***

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Original Article

## Ecological and Biological Insights into *Cuscuta planiflora*: A Fundamental study exploring its Morphology and Parasitic Behavior on *Rhanterium epapposum*

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### Abstract

The parasitic plant *Cuscuta planiflora*, commonly known as dodder, exhibits specialized adaptations that enable its survival and reproduction by exploiting host plants. One such host is *Rhanterium epapposum*, a native shrub of the Arabian Peninsula, important for grazing and locally known as Arfaj. The susceptibility of *R. epapposum* to parasitism by *C. planiflora* was investigated in Abdali farmland. Observations indicated that the vegetation of *R. epapposum* decreased in areas with sandy soil, where *C. planiflora* was absent. In contrast, areas with loamy soil supported more robust growth of *R. epapposum*, but many of these plants were parasitized by *C. planiflora*. This study highlights the influence of soil type on the interaction between *C. planiflora* and *R. epapposum*, with sandy soils being less conducive to parasitism, thereby affecting the distribution and health of *R. epapposum* communities. This study explores the phenology, morphology, ecology, and host-parasite interactions between *C. planiflora* and *R. epapposum*. It aims to offer a comprehensive synthesis of existing knowledge while identifying key areas for future research.

## 1. Introduction

All living organisms on the earth can be classified according to the way of nutrition to heterotroph, autotroph, or mixotroph. All green plants (approximately 380,000 species) are in Kingdom Plantae, including all multicellular photosynthetic (obtain energy from water, carbon dioxide, and minerals) organisms (autotroph). Angiosperms represent the vast majority of the Kingdom (approximately 300,000 species) (Hibberd & Jeschke, 2001; Johnson et al., 2016; Watson, 2009). However, the exception of that is parasitic plants (approximately 4,000 species) belong to angiosperms; they are either partially or fully dependent on photosynthetic plants to obtain nutrients such as carbon and nitrogen compounds; also known as xylem-feeding plants (Hibberd & Jeschke, 2001; Watson, 2009).

The parasitic plants acquire water, nutrients, and carbon sources from the host's root and stem using a specialized organ called haustoria (modified stems or roots). Parasitic plants penetrate their hosts' xylem and phloem tissues (vascular system) through

haustoria to make a bridge connection between both the host and the parasite. Therefore, the connection between both plants' vascular systems allows the parasite to obtain solutes, organic acids, and water from the xylem tissues, while the sugars and amino acids are obtained from host phloem tissues (Albert et al., 2008; Birschwilks et al., 2006; Bleischwitz et al., 2010; Furuhashi et al., 2011; Hibberd & Jeschke, 2001).

The parasitism phenomena in the Plant Kingdom is thought to be an evolution of flowering plants through several stages (Albert et al., 2008; Furuhashi et al., 2011; Hibberd & Jeschke, 2001). The relationship varies between parasitic plant species, including species containing chlorophyll pigment, which allows photosynthate and parasitism, known as facultative parasites. In addition, obligate parasites are parasitic plants that fully rely on the host and cannot produce sugar via photosynthesis due to lacking pigments and enzymes (Furuhashi et al., 2011). Hence, the differences between parasitic plants include

anatomy and morphology of it, such as having roots and leaves. Among all parasitic species in the 17 families, some of these plants have functional roots such as *Rhinanthus* sp. and *Oxalis* sp. which are capable of taking up water and nutrients directly from soil. While others, such as Cuscutaceae and mistletoes (parasitic plants of order Santalales), lack roots (Hibberd & Jeschke, 2001; Reville et al., 2005).

Cuscutaceae is belonging to the Convolvulaceae family, comprising of 200 species under a single genus *Cuscuta* (dodders) (Furuhashi et al., 2011; Olsen et al., 2016). The *Cuscuta* sp. has a broad geographical and host range implemented on crop production. *Cuscuta* sp. may cause a reduction in crop yields, for example, around 60% in alfalfa, 50-75% in tomato, and 70-100% in carrot and cranberry (Johnson et al., 2016). Furthermore, *Cuscuta* sp. lack of the photosynthetic chlorophyll pigment that required for assimilate atmospheric CO<sub>2</sub> such as *Cuscuta gronovii*, while some *Cuscuta* species such as *Cuscuta reflexa* contain fewer thylakoid in their plastids compared to normal angiosperm plastids allowing the plant to limit photosynthetic activities (Albert et al., 2008; Birschwilks et al., 2006; Funk et al., 2007a, 2007b; Haupt et al., 2001a).

*Cuscuta planiflora* is characterized by its thin, thread-like, twining stems that appear yellow or orange. Lacking leaves, the plant's stems are covered with small scale-like structures. Flowers are typically small and grouped in inflorescences (Noureen et al., 2019). The absence of chlorophyll in *C. planiflora* necessitates its parasitic mode of life, where the plant derives all its required nutrients from the host.

Ecologically, *C. planiflora* plays a significant role in shaping plant communities by targeting various host species. It prefers abundant and nutritionally rich hosts, often selecting dominant species within a community, which may lead to decreased host plant competitiveness and altered community structures (Fürst et al., 2016; Furuhashi et al., 2011a; Hartenstein et al., 2023; (Shimizu & Aoki, 2018, 2019)). The presence of *C. planiflora* can reduce biodiversity if dominant species are significantly weakened, allowing less competitive species to increase in prevalence. The ecological impact of *C. planiflora* extends to various ecosystems where it can influence the diversity and vitality of plant communities—understanding the life cycle, structural adaptations, and ecological relationships of *C. planiflora* is crucial for managing its impact in agricultural and natural ecosystems. The interaction

between *C. planiflora* and its hosts involves physical penetration and biochemical interactions. The haustoria not only physically breaches the host's xylem and phloem to access nutrients but also involves a sophisticated exchange of molecular signals. These signals may suppress the host's defensive responses, allowing the parasite to evade detection and continue extracting resources unimpeded. Hosts vary in their susceptibility to *C. planiflora*, with some capable of launching effective defensive mechanisms, such as tissue necrosis around the penetration site, effectively cutting off the parasite's nutrient supply (Hartenstein et al., 2023; Shimizu & Aoki, 2019 ).

In Kuwait, only a single *Cuscuta* species is listed in the Kuwaiti flora represented by *Cuscuta planiflora* (locally known as Arooq, Shubakah, Hamool) (Boulos & Al-Dosari, 1994; Omar, 2007). During the spring season, it grows on desert perennial shrubs such as *R. epapposum* (Arfaj) and *Lycium shawii* (Awsaj), affecting native plants and reducing the vegetation coverage. In addition, it is found in local farms, reducing the crop yield productivity of *Solanum lycopersicum* (tomato) and *Medicago sativa* (alfalfa). Further research is required to study *C. planiflora* and its morphological and parasitic behavior in relationship with different hosts and understand how to control its behavior.

This study's primary purpose is to understand better the vital function of parasitic plant and its behavior on native plants in arid ecosystems. The main objectives are as follows: 1) to determine the impact of *C. planiflora* on the *R. epapposum* community, and 2) to investigate the physical and chemical properties of the soil in high vegetation (HV) and low vegetation (LV) plots and how they limit *Cuscuta* growth and development.

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## **2. Materials and Methods**

### **2.1 Study area:**

The study area is fence protected in AlAbdali agricultural area of size 200,000 m<sup>2</sup> (Northern part of Kuwait). AlAbdali area receives very low rainfall, around 121.1 mm of precipitation annually. The average temperature range between 44 to 49 °C in summer and the highest temperature recorded was 52.8 °C. The average minimum temperature range between 3 to 12 °C, and the lowest temperature recorded was -2.9 °C. The dominant vegetation in the area is *R. epapposum* community, a native perennial shrub. Parts of the field are covered with high vegetation of *R. epapposum* and other parts with lower vegetation. The study plots were divided into High Vegetation (HV)

and Low Vegetation (LV) plots based on previous work using Remote Sensing (RS) and Geographic Information System (GIS) techniques (Abdullah et al., 2021; Abdullah et al., 2024 ).

**2.2 Field survey:**

**2.2.1 Quantifying R. epapposum:**

To study the R. epapposum vegetation cover, measurements of eight line-transects of length 50 m were conducted inside four plots of size 50X50 m (two plots represent HV cover and two plots LV cover). The height and canopy size of each R. epapposum were recorded. In addition, the appearance of C. planiflora was recorded on each R. epapposum individual along the line-transect.

**2.2.2 Soil Sample collection and analysis**

For the comparison of soil and vegetation cover, 16 soil samples were collected from the study plots representing HV and LV. The soil samples were collected from 5 to 20 cm depth (below the surface) for further analysis of the soil's properties. The physical analysis of the soil was measured to determine the type of soil present (sandy, clay, silty, or loamy soils). Furthermore, soil chemical parameters were determined: electrical conductivity (EC), soil moisture, nitrate (NO<sub>3</sub>-), Carbon (C), pH, and total phosphorus (P).

**2.3 Plant sampling:**

Rhanterium epapposum and C. planiflora plant samples were collected from the field and examined in the Lab under a stereo microscope (Type: Nikon SMZ75T). Images were captured using a fitted digital camera (Type: Nikon DS-Fi3). The imaging process application used was NIS-Elements imaging software by Nikon.

**2.4 Data analysis and statistics:**

The statistical analysis (t-test and comparison of group means) assessment was conducted using GraphPad Prism (version 10, 2023). To perform the t-test analysis, the soil data was imported and grouped by sample location. An unpaired t-test was then applied to compare means between groups, with settings adjusted for equal variances. Results included means, p-values, confidence intervals, and standard deviations, with a p-value of less than 0.05 indicating statistical significance.

The following hypothesis was conducted to test the significant level of the correlation of C. planiflora on the growth of R. epapposum, using soil properties analysis: If the chemical and physical qualities of soil parameters representing HV and LV plots vary. If this case is valid, this investigation will show a strong interlinkage between the existence of C. planiflora

parasitism and R. epapposum vegetation cover. In other words, soils containing high vegetation cover have richer soil qualities (i.e., nutrients, moisture, and grain size variety).

**3. Results**

**3.1 Rhanterium epapposum Vegetation Cover**

The study area is mainly dominated by a native perennialshrub, R. epapposum. R. epapposum (Fig.1) vegetation cover ranges between 8.5% (LV plots) to 50% (HV plots) in the study plots. The newly grown stem of R. epapposum showed a white color while the older branches are brownish and woody (Fig. 2).

**Table 1.** soil analysis in area with high vegetation (HV) and low vegetation of R. epapposum.

Vegetation cover of R. epapposum	healthy Rhanterium (No.)	Rhanterium Width (cm)	Rhanterium Length (cm)	No. of Rhanterium with Cuscuta	Rhanterium + Cuscuta Width (cm)	Rhanterium + Cuscuta Length (cm)
High Vegetation (HV)	20	59 - 74	62 - 90	12	45 - 61	50 - 60
Average		66	76		53.3	54.3
Low Vegetation (LV)	13	22 - 45	13.4 - 55.9	0	-	-
Average		34	34.7			

In the studied plots, R. epapposum is the most susceptible host parasitized by C. planiflora compared to other annuals and perennials present in the area (Fig. 3 A and C). The aggressive parasitism behavior as shown in (Fig. 4) caused a decline in vegetation cover of R. epapposum growth in LV plots (Table 1). While HV plots showed an increase in vegetation cover of R. epapposum in the absence of C. planiflora (Table 1).

Table 1 showed that the parasitic plants can be found only in high density of vegetation which can be determined clearly.

**Table 2:** The analysis of different soil variables in rich vegetation plot (HD), and low vegetation plot (LD). Values represent mean ± SEM, and number of samples (n).

	EC (µmho/cm)	Nitrate (mg/kg)	Nitrite (mg/kg)	pH	phosphorus (mg/kg)
HV	12.94 ± 4.92 n = 9	3.48 ± 1.2 n = 9	0.13 ± 0.10 n = 9	7.9 ± 0.12 n = 9	492.6 ± 1030 n = 9
LV	24.66 ± 19.9 n = 8	2.19 ± 1.45 n = 8	0.08 ± 0.04 n = 8	7.7 ± 0.3	121 ± 21.0 n = 8

Furthermore, vegetative growth in the area showed that soil properties influence R. epapposum vegetation cover richness, and the appearance of Cuscuta planiflora is linked to the soil parameters represented by HV and LV plots in Table 2.

### 3.2 Cuscuta behaviour and observation

The parasitism of *C. planiflora* on *R. epapposum* was seen in the plots, observed by a yellow to red stem (Fig. 3C), white flower (Fig. 5) and wind itself on all vegetative parts of *R. epapposum* causing a reduced growth of *R. epapposum*.

**Table 3:** The analysis of different soil variables in rich vegetation plot (HD), and low vegetation plot (LD). Values represent (mean ± SEM), and number of samples (n), asterisks (\*\*\*) indicate significance  $P < 0.001$ .

	EC (µmho/cm)	Nitrate (mg/kg)	Nitrite (mg/kg)	pH	phosphorus (mg/kg)
<b>H V</b>	12.94 ± 4.92 n = 9	3.48 ± 1.2 n = 9	0.13 ± 0.10 n = 9	7.9 ± 0.12 n = 9	492.6 ± 1030 n = 9
<b>LV</b>	24.66 ± 19.9 n = 8	2.19 ± 1.45 n = 8	0.08 ± 0.04 n = 8	7.7 ± 0.3	121 ± 21.0 n = 8

The parasitic growth of *C. planiflora* emerges to find a compatible host. It runs on the soil surface using the grass plant *Stipellula capensis* as a connecting bridge and makes a network-like of *Cuscuta* stem to find its nearest susceptible host (Fig. 6). Once a preferable host is found then the *C. planiflora* starts to twist on it and produce a parasitic organ known as haustoria (Fig. 7). Haustoria is the connection point between parasitic plants and their hosts. After that, it starts to induce flowering before the end of the spring season.



**Figure 1. *R. epapposum* shrub in the study area**

### 3.3 Soil Properties analysis

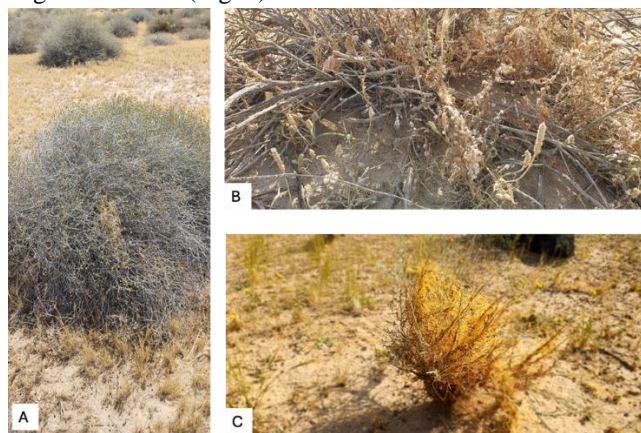
From the measured data represented in Table 2 results showed that the area with rich vegetation contains more loamy soil compared to sandy soil in areas with low vegetation cover.



**Figure 2. Showing yellow flowers and young white woolly stem of *R. epapposum*.**

The different soil nutrient minerals and molecules analysis did not showed any significant difference by applying t – test, between two variables the high - density

(HD) plots and Low - density (LD) *R. epapposum* plots as indicated in (Table 2). The Electrical conductivity (EC) and pH scale also did not reveal any significant relationship between low density and high-density vegetation areas (Fig. 8).



**Figure 3. *C. planiflora* parasitism on *R. epapposum*; gradually shows vegetation cover loss. A and C, showing growth of *C. planiflora* on *R. epapposum* in the field. B, flowers of *C. planiflora* clear while growing on annual plant such as *Plantago boissiri*.**

Hydrogen ions concentration in the soil showed very highly significant in low density (LD) *R. epapposum* plots (Table 3). Otherwise, other variables did not point out any significant in the two types of vegetation plots (Table 3).



**Figure 4. *Cuscuta planiflora* grow on *R. epapposum* in heavy network of growth.**

The soil texture analysis showed large area of the means for Clay and Silt in High density vegetation plots (HD) as shown in Table 2. On the other hand, the sand and gravel were more common in low vegetation plots (LD).



Figure 5. *C. planiflora* flower.



Figure 6. *C. planiflora* grow above ground and use the grasses as bridge to reach the preferred host.



Figure 7. The *C. planiflora* grow, twist around *R. epapposum*. a, The flower of *R. epapposum* is yellow. The growth of *C. planiflora* stem and produces the white flower. c: *C. planiflora* twisted and coiled around new season *R. epapposum* stem which is green in color covered by white hair. d: The haustorium points on the stem is noticed.

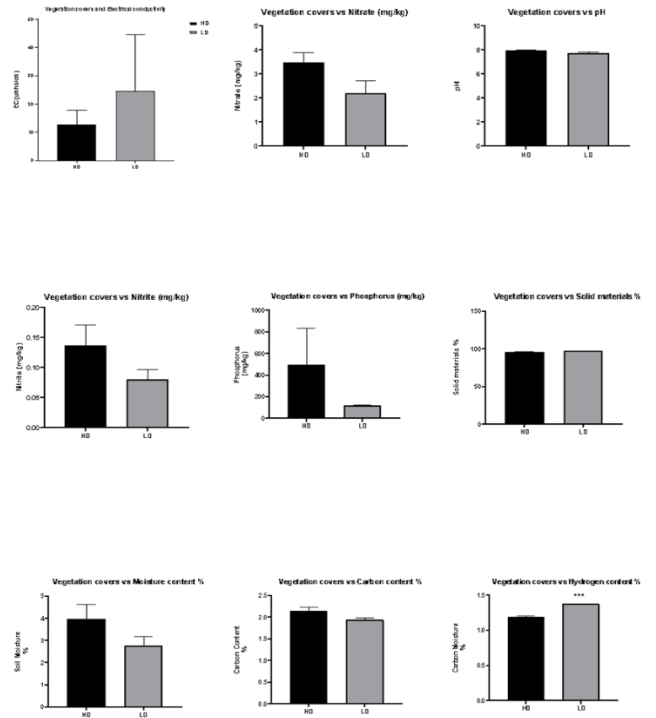


Figure 8. Comparison between vegetation cover plots (Mean ± SEM), with high - density plants (HD) and low - density plants (LD), with different soil analysis parameters. The relationship was only significant with Hydrogen % soil analysis  $P < 0.001$ .

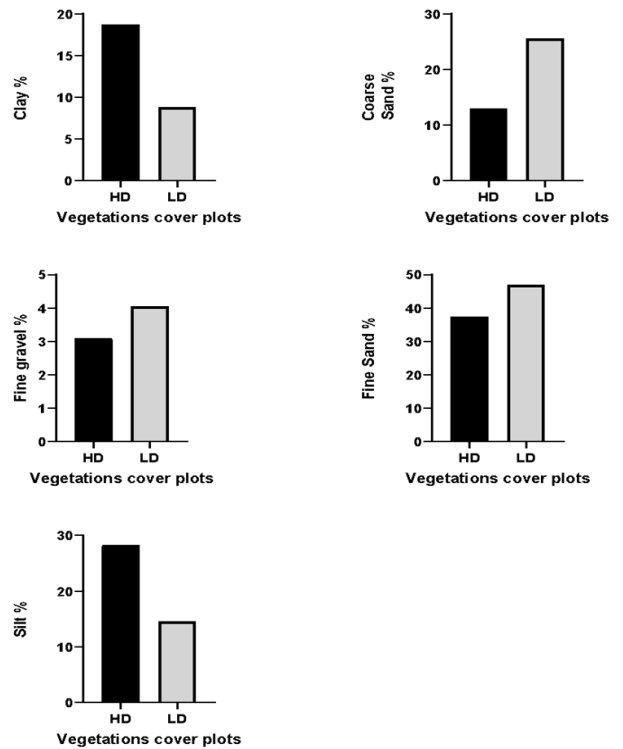
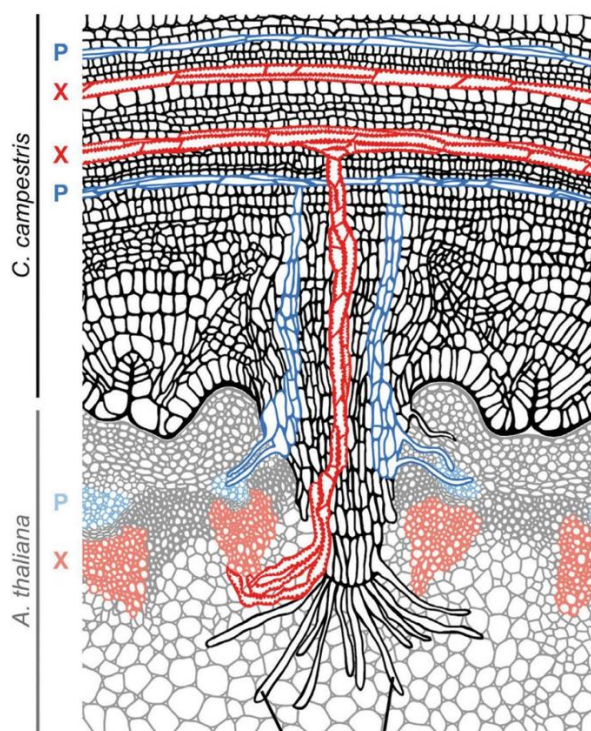


Figure 9: The mean percentages of different soil texture on plots with high - density vegetation cover (HD) and low-density vegetation cover (LD).



**Figure 10. Schematic diagram on how haustoria penetrate the vascular tissues. Adopted from (Hartenstein et al., 2023)**

#### 4. Discussion

The growth of parasitic plants in Kuwait need to be more identified. *Cuscuta*. sp. is an obligate parasite that lack of root (Hibberd & Jeschke, 2001; Revill et al., 2005), holoparasitic (Bernal-Galeano et al., 2022; Birschwilks et al., 2006; Evans & Borowicz, 2013 ; Haupt et al., 2001b) that obtained all requirement to survive, growth and reproduce from host plant. The observation in the field shows that *Cuscuta* parasitized on annuals plant such as *Plantago boissiri* and other annuals.

The *Cuscuta* starts to germinate and find nearest preferred host which is *R. epapposum* in this study. Start by induction of near far-red light which is used by *C. planiflora* to find the preferred host usually flowering plants, further more *C. planiflora* start to twist and coil around leaf and stem of the host (Haidar & Orr, 1999), Acquiring nutrients and water by producing haustoria to penetrate stem or leaf of its host plant.

Then, haustoria initiated (Kaga et al., 2020; Shimizu et al., 2018; Shimizu & Aoki, 2018) to obtain nutrients and water. The haustorium contains searching hyphae to find vascular tissues as mentioned in the figure 9.

Results of this study found that, *C. planiflora* preferring *R. epapposum* in the field among all other shrubs. The host range of *Cuscuta* sp. is large while

in some cases prefers other hosts due to different reasons such as enhancing growth of *Cuscuta*, signaling between host and parasite, or might be other reasons (Kaga et al., 2020; Kaiser et al., 2015; Olsen et al., 2016; Press & Phoenix, 2005).

The various soil types showed no discernible effects on the dodder plants (*C. planiflora*), neither in the High-vegetation plots (HV) nor in the Low-vegetation plots (LV). The explanation is because *Cuscuta* plants lack roots in their taxonomic structure and do not rely on soil nutrients for germination or survival. Moreover, soil elements are not necessary for the germination of their seeds (Furuhashi et al., 2011). Furthermore, a survey of holoparasitic plants in Jordan revealed that *Cuscuta* spp. were prospering in both rainfed clay soil and irrigated sandy soil (Qasem, 2008). In light of this, recent study soil results support earlier research.

The findings, with regard to *R. epapposum*, verify that the Arfaj soil range was formed with high silica and clay levels. *R. epapposum* community of plants exists in area with deep and loamy soil, according to Omar et al., (2001) investigation of Kuwait's native vegetation. Furthermore, an ecological plants assessment conducted in Saudi Arabia's Al-Ammaria Wadi, in the country's northwest, confirmed that the Arfaj plants had been developed in soil that contained at least 14% clay (Al-Ammaria et al., 2017). As a result, our research confirms that *R. epapposum* Favors deep soil that has a high silt and clay concentration.

#### Conclusion

*Cuscuta planiflora* represents a fascinating example of evolutionary adaptation to a parasitic lifestyle. Its impact on host plants and plant communities underscores the importance of understanding its biology and ecology for effective management in both agricultural and natural ecosystems. Future research should focus on the molecular interactions between *C. planiflora* and its hosts, as well as strategies for managing its spread in sensitive or economically important habitats.

This study synthesizes the current understanding of *C. planiflora*, emphasizing its role as an ecological agent and its interactions within various plant communities. As research continues, more detailed insights are expected, particularly in the areas of molecular biology and ecological impact management.

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