Host Associations of Gall-Inducing Prodiplosis longifila (Diptera: Cecidomyiidae) from Bolivia: Implications for Its Use as a Biological Control Agent for Jatropha gossypiifolia (Euphorbiaceae)

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Host associations of gall-inducing *Prodiplosis longifila* (Diptera: Cecidomyiidae) from Bolivia: implications for its use as a biological control agent for *Jatropha gossypiifolia* (Euphorbiaceae)

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Abstract

Based on field host range and damage potential, we explored the prospects of exploiting a gall midge from *Jatropha clavuligera* Müll. Arg. (Euphorbiaceae) in Bolivia as a "new-association" biological control agent for *Jatropha gossypiifolia* L. (Euphorbiaceae), a major rangeland weed in Australia. The gall midge, determined morphologically as *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae), induces rosette galls, resulting in shoot tip dieback in *J. clavuligera* in Bolivia. Although *P. longifila* is reported to occur on a range of crops in the Neotropics, its feeding on *J. clavuligera* in Bolivia is strikingly different in that it induces rosette galls on its shoots. In other countries, *P. longifila* larvae feed on leaves, buds, flowers and fruits, with no gall symptoms. There was no evidence of *P. longifila*-induced galls on crop plants (reported hosts in other countries), other species of *Jatropha*, or other species of Euphorbiaceae in Bolivia, and in choice tests conducted in the quarantine facility in South Africa. Bolivian entomological records do not report any damage to crops by *P. longifila*. We propose that *P. longifila* in Bolivia is possibly a new species restricted to a few closely related species of *Jatropha* and a part of a cryptic species complex. Based on the susceptibility of *J. gossypiifolia* to the gall-inducing *P. longifila* in the field in Bolivia and in quarantine tests in South Africa, we propose that the gall-inducing *P. longifila* sourced from *J. clavuligera* in Bolivia is a potential "new-association" biological control agent for *J. gossypiifolia* in Australia.

Key Words: Gall midge; field host range; host specificity; cryptic species; native-range survey; weed biological control

Resumen

Sobre la base del rango de hospederos de campo y el potencial de daño, exploramos las perspectivas de la explotación de un mosquito de agalla de *Jatropha clavuligera* Müll. Arg. (Euphorbiaceae) en Bolivia como un agente de control biológico de "nueva asociación" para *Jatropha gossypiifolia* L. (Euphorbiaceae), una importante maleza de pastizales en Australia. El mosquito de agalla, identificado morfológicamente como *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae), induce las agallas de la roseta que dan lugar a la desintegración de la punta del brote en *J. clavuligera* en Bolivia. Aunque se informe que *P. longifila* ocurre sobre una variedad de cultivos en el Neotrópico, su alimentación en *J. clavuligera* en Bolivia es sorprendentemente diferente en cuanto que induce agallas de roseta en sus brotes. En otros países las larvas de *P. longifila* se alimentan de hojas, capullos, flores y frutos, sin síntomas de agallas. No hubo evidencia de agallas inducidas por *P. longifila* en plantas de cultivo (reportadas en otros países), otras especies de *Jatropha* y otras especies de Euphorbiaceae en Bolivia y en pruebas de selección realizadas en la instalación de cuarentena en Sudáfrica. Los registros entomológicos bolivianos no reportan ningún daño a los cultivos por *P. longifila.* Proponemos que *P. longifila* en Bolivia es posiblemente una especie nueva restringida a algunas especies estrechamente relacionadas de *Jatropha* y una parte de un complejo de especies crípticas. Basado en la susceptibilidad de *J. gossypiifolia* a la *P. longifila* que induce agallas en el campo en Bolivia y en las pruebas de cuarentena en Sudáfrica, proponemos que la *P. longifila* induce agallas procedente de *J. clavuligera* en Bolivia es una "nueva asociación" y un potencial agente de control biológico de *J. gossypiifolia* en Australia.

Palabras Clave: mosquito de agallas; rango de hospederos del campo; especificidad del hospedero; especies crípticas; sondeo de rango nativo; control biológico de malezas

Classical weed biological control involves the importation and establishment of self-perpetuating, host-specific natural enemies from the native landscape of the target weed. Host-specific natural enemies are usually sourced from the same target weed in the native landscape (e.g., Goolsby et al. 2006; McFadyen 1998). However, natural enemies from congeners also have been successfully used as biological con-

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trol agents to manage weeds (e.g., Dodd 1940; Julien 2012; Palmer & McFadyen 2012; Palmer & Sims-Chilton 2012; van Klinken & Morin 2012). In this paper, we report the exploration of a similar strategy of using agents from *Jatropha clavuligera* Müll. Arg. (Euphorbiaceae), as a "new-association" biological control agent for *Jatropha gossypiifolia* L. (Euphorbiaceae), a weed of national significance in Australia.

Jatropha gossypiifolia, a deciduous perennial shrub native to tropical America, is a major and expanding weed of rangelands and riparian zones in dry tropical parts of northern Australia (Bebawi et al. 2007). *Jatropha gossypiifolia* forms dense thickets, reducing the usefulness of land for grazing by competing with pasture species and poisoning stock. It also reduces biological diversity, affects fire regimes, and increases erosion along creek and river banks (Csurhes 1999, Bebawi et al. 2007). Biological control is the most economically viable and long-term management solution for *J. gossypiifolia* (Dhileepan et al. 2014).

Native-range surveys in Mexico, Central and northern South America, and the Caribbean for potential biological control agents resulted in the release of seed-feeding *Agonosoma trilineatum* (Fab.) (Hemiptera: Scutelleridae) in Australia, which failed to establish (Heard et al. 2012). With no prospects of gaining any additional biological control agents from Mexico, Central America, and the Caribbean (Heard et al. 2012), survey efforts were redirected to South America (Dhileepan et al. 2014). Because all *J. gossypiifolia* in South America (e.g., Bolivia, Paraguay, Peru, and Brazil) were found either in home gardens or as garden escapes (Dhileepan et al. 2014), other *Jatropha* species native to South America also were surveyed for specialist natural enemies with their host range limited to a few closely related *Jatropha* species. With no native *Jatropha* species in Australia and with *Jatropha* species not regarded as major ornamentals or crops, use of a host-specific agent from congeners as a "new-association" biological control agent for *J. gossypiifolia* in Australia is a promising option.

Surveys in Bolivia revealed populations of *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae), which induce rosette galls on the shoot terminals of *J. clavuligera*, a close relative of *J. gossypiifolia*, in Bolivia, resulting in shoot-tip dieback (Dhileepan et al. 2014). *Prodiplosis longifila* has been reported as a pest of various crops such as citrus (*Citrus* spp., Rutaceae), asparagus (*Asparagus officinalis* L., Asparagaceae), alfalfa (*Medicago sativa* L., Fabaceae), potato (*Solanum tuberosum* L., Solanaceae), tomato (*S. lycopersicum* L., Solanaceae), bean (*Phaseolus* spp., Fabaceae), peppers (*Capsicum* spp., Solanaceae), wild cotton (*Gossypium* sp., Malvaceae), artichoke (*Cynara cardunculus* L., Asteraceae), cucurbits (*Cucurbita* spp., Cucurbitaceae), avocado (*Persea americana* Mill., Lauraceae), onion (*Allium cepa* L., Amaryllidaceae), grapevine (*Vitis vinifera* L., Vitaceae) and castor-oil plant (*Ricinus communis* L., Euphorbiaceae) in the Neotropics (Gagné 1986; Peña et al. 1989; Gagné & Jaschhof 2014). However, gall induction on *J. clavuligera* and the field host range of *P. longifila* in Bolivia suggest that the populations of *P. longifila* inducing galls on *J. clavuligera* in that country belong to a host-specific, cryptic species within the *P. longifila* species complex. The *P. longifila* populations living on other plants are possibly a complex of cryptic species, rather than a single polyphagous species (EPPO 2015). Similar morphologically indistinguishable cryptic species pose significant challenges in other weed biological control projects (Mound et al. 2010; Toševski et al. 2011, 2013; Rafter et al. 2013; Paterson et al. 2016).

In this study, we explored the feasibility of exploiting the gall-inducing *P. longifila* sourced from *J. clavuligera* (the native host) in Bolivia as a biological control agent for *J. gossypiifolia* (the novel host) in Australia, based on the incidence, damage levels, and field host range of gall-inducing *P. longifila* on *J. clavuligera* in Bolivia, and on the susceptibility of *J. gossypiifolia* to *P. longifila* gall damage in a field transplant experiment in Bolivia and in no-choice tests in quarantine in South Africa.

Materials and Methods

NATIVE-RANGE SURVEY

Based on herbarium records of the Missouri Botanical Garden, Kew Royal Botanic Gardens, National Herbarium Nederland, New York Botanical Garden, Rio de Janeiro Botanical Garden, and the Harvard University Herbarium, and related published literature (Dehgan 2012), surveys were conducted on *J. gossypiifolia*, *J. clavuligera*, *J. excisa* Griseb., *J. hieronymii* Kuntze and *J. curcas* L., at 13 sites in Bolivia in Apr 2013, Nov 2014, Mar 2015, and Feb 2016 (Table 1). *Jatropha gossypiifolia* and *J. curcas* found in Bolivia were cultivated in home gardens, suggesting that these 2 species are not native taxa here. Other Bolivian native species of *Jatropha*, such as *J. clavuligera*, *J. excisa*, and *J. hieronymii* occurred mainly along road verges, in conservation areas, and arid-zone forests. The incidence (measured as percentage of plants) of *P. longifila* galls on *J. clavuligera*, *J. gossypiifolia*, *J. excisa*, *J. curcas*, and *J. hieronymii* was recorded at all sites during sampling. Because *P. longifila* galls were found only on *J. clavuligera*, the percentage of *J. clavuligera* shoot tips with galls was recorded at 3 sites (Pulquina Cacti Garden, San Isidro, and La Villa near Punata; Table 1) in Feb 2016. The field-collected galls were dissected out to determine if there were live or developing larvae, parasitoids, and also to verify possible fungal associations within galls. The larvae of *P. longifila* extracted from galls were reared to adults in a quarantine facility at the Agricultural Research Council–Plant Protection Research Institute (ARC–PPRI) in Pretoria, South Africa. Alcohol-fixed *P. longifila* specimens were sent to Raymond Gagné (USDA-ARS) for determination.

LIGHT MICROSCOPY

Galled shoots of *J. clavuligera* were spot fixed in FAA [formalin, ethanol (95%), glacial-acetic acid, distilled water:10, 50, 5, 35 ml, making up to 100 ml], followed by processing in alcohol series (30, 50, 70, 80, 90, 100%, each change 12 h), histolene, and paraffin-wax embedding (65 °C). The wax-embedded tissues were sectioned at 8 μm using a rotary microtome, deparaffinized in histolene, contrasted with 1% toluidine blue (in 1% aqueous-borax solution), and mounted in DPX. Micrographs were made in a photomicroscope (BX-51, Olympus Optical Co., Ltd., Tokyo, Japan). Staining with toluidine blue (1%) also localized phenolic materials (McCully 1966).

PRELIMINARY MULTIPLE AND NO-CHOICE TESTS IN QUARANTINE

Field-collected *P. longifila* galls and young, bare-rooted (uprooted seedlings with soil washed off from roots and roots wrapped with moist paper towel) *J. clavuligera* plants bearing *P. longifila* galls were imported into the ARC-PPRI quarantine facility in Pretoria, South Africa, in Nov 2014. Field-collected *J. clavuligera* plants with early symptoms of gall development were transplanted into pots in the quarantine facility and the larvae were allowed to mature and pupate in the soil. The emergent *P. longifila* adults (3 to 4 adults per d over a wk) were exposed to potted ungalled *J. gossypiifolia* control plants maintained individually in insect-proof cages (50 cm × 50 cm × 100 cm; 1 *J. gossypiifolia* plant per cage), under no-choice conditions, to verify whether gall-inducing *P. longifila* obtained from *J. clavuligera* in Bolivia induced galls also on *J. gossypiifolia*. Simultaneously, the emergent *P. longifila* adults (3 to 4 adults per d over a wk) were exposed to potted ungalled *J. clavuligera* plants maintained individually in insect-proof cages (50 cm ✕ 50 cm ✕ 100 cm; 1 *J. clavuligera* plant per cage), under no-choice conditions, as control plants to compare the nature of galls induced on *J. clavuligera*, the native host, and on *J. gossypiifolia*, the novel host. Both *J. gos-*

Table 1. Incidence and abundance of *Prodiplosis longifila* galls or larval feeding damage on *Jatropha* species, closely related Euphorbiaceae species and crops at survey sites in Bolivia.

| | | | Plants with midge galls/total number of plants sampled | | | | | | | | | |
| | | | Genus *Jatropha* | | | | | Euphorbiaceae | | Crops | | |
Location	Latitude and longitude	Month-Year	*Jatropha clavuligera*	*Jatropha excisa*	*Jatropha hieronymii*	*Jatropha gossypiifolia*	*Jatropha curcas*	*Cnidosculus tubulosus*	*Ricinus communis*	Tomato	Potato	Orange
Santa Cruz	17.8714°S, 63.1316°W	Nov-2014	N/A			0/7			0/5			
Piray river	18.1091°S, 63.4578°W	Apr-2013	N/A	0/3								
		Nov-2014	N/A	0/4								
Angostura	18.1567°S, 63.4994°W	Apr-2013	N/A			0/4	0/3		0/8			
		Nov-2014	N/A			0/4	0/3					
		Mar-2015	N/A			0/4	0/1		0/5			
Mairana	18.1573°S, 63.9436°W	Apr-2013	N/A				0/1		0/5			
		Nov-2014	N/A				0/1					
Los Negros	18.0443°S, 63.1176°W	Apr-2013	N/A	0/11			0/1					
		Nov-2014	N/A	0/10			0/1					
Pambagrande	18.0756°S, 64.1203°W	Apr-2013	N/A	0/6					0/4			
		Nov-2014	8/11						0/12			
Camino Pulquina Areva	17.9777°S, 64.4749°W	Apr-2013	4/21									
		Nov-2014	0/9									
San Isidro	18.0122°S, 64.4594°W	Apr-2013	8/13									
		Nov-2014	3/18									
		Mar-2015	6/22						0/100			
		Feb-2016	81/98						0/20	0/20	0/50	
Puliquna cacti garden	18.0997°S, 64.4256°W	Apr-2013	9/47									
		Nov-2014	33/38									
		Mar-2015	16/19									
		Feb-2016	52/66					0/20	0/10	0/20		0/10
Comarapa - Dam	17.9408°S, 64.5616°W	Apr-2013	9/13									
Saipina	18.0975°S, 64.5917°W	Apr-2013	N/A	0/8	0/5							
La Villa, Punata	17.5122°S, 65.8113°W	Apr-2013	0/19									
		Mar-2015	18/24						0/10	0/10		
		Feb-2016	92/100									
Arani, Cochabamba	17.5837°S, 65.7563°W	Apr-2013	0/44						0/10			0/8
		Mar-2015	2/34									

sypiifolia and *J. clavuligera* control plants without galls exposed to *P. longifila* adults were monitored for gall development.

Jatropha clavuligera without galls, together with combinations (4 test plants per cage) of tomato, bell pepper (*Capsicum annuum* L.), castor-oil plant, lemon (*Citrus limon* [L.] Burm.f.), *J. gossypiifolia*, *J. curcas* and 4 Euphorbiaceae native to southern Africa, viz., *J. zeyheri* Sond., *Croton gratissimus* Burch., *Synadenium cupulare* (Boiss.) L.C. Wheeler, and *Flueggia virosa* (Willd.) Voigt., were included in choice tests for oviposition and gall induction by *P. longifila*. These trials were conducted in insect-proof cages in a quarantined glasshouse in Pretoria, South Africa. Newly emerged females and males of *P. longifila* were introduced into insect-proof cages with batches of test plants, over a period of up to 1 wk, as they became available from field-collected galls (3 to 4 adults per d over a wk). Due to limited number of female emergence, no replications were possible in the preliminary choice tests (1 replication for each test plant species). The plants were observed for morphological changes in shoot tips and gall development for 2 wk after the last gravid female was introduced into each cage.

FIELD TRANSPLANT EXPERIMENTS

To ascertain the susceptibility of *J. gossypiifolia* to gall induction by *P. longifila* under natural field conditions, 16 field-collected *J. gossypiifolia* seedlings from Santa Cruz (17.5200°S, 63.0700°W) and Angostura (18.0900°S, 63.2900°W) were planted around 4 *J. clavuligera* plants bearing galls induced by *P. longifila*. The 4 *J. gossypiifolia* seedlings were planted 60 cm apart from each *J. clavuligera* plant along the 4 compass points at the Pulquina Cacti Garden (18.0500°S, 64.2500°W) near Comarapa in Bolivia in Nov 2014. In addition, 20 seedlings and 5 stem cuttings of *J. gossypiifolia* also were planted individually in polybags and maintained in the shade under a randomly selected tree at the Pulquina Cacti Garden. The susceptibility of *J. gossypiifolia* plants (16 transplanted and 25 in polybags) to gall induction by *P. longifila* was checked in Mar 2015 and Feb 2016.

Because the Cecidomyiidae on *J. clavuligera* had been identified as *P. longifila* (Raymond Gagné, personal communication), a field transplant experiment was initiated at the Pulquina Cacti Garden in Mar 2015 to check whether *P. longifila* will induce galls on those plants reported as hosts for *P. longifila* in countries other than Bolivia. Four plots (9 m² each) with mature *J. clavuligera* plants with *P. longifila* gall damage were used. In each plot, 3 to 4 *J. clavuligera* plants bearing galls induced by *P. longifila* were identified as the source plants. Tomato, potato, bell pepper, orange, and castor-oil plants (reported hosts for *P. longifila*) and *J. clavuligera* as control were transplanted at 60 cm distance from each *J. clavuligera* plant with *P. longifila* galls in a split-plot design (6 test plant species × 3 replications × 4 plots). The experiment was checked for freshly induced galls on transplanted *J. clavuligera* and the 5 economically important plant species in Feb 2016.

FIELD-HOST RANGE

The crops and other plants reported as hosts for *P. longifila* in the USA (Florida), Peru, Ecuador, and Colombia, were surveyed in Bolivia for either *P. longifila* incidence or its damage symptoms (Table 1). In Apr 2013, the castor-oil plant (reported host of *P. longifila*), *J. gossypiifolia*, *J. curcas*, and *J. excisa* occurring along road verges were sampled at 6 sites (Table 1). In Nov 2014, *J. excisa*, *J. curcas*, and *J. hieronymii* were sampled at 5 sites (Table 1). In Mar 2015, orange trees in 2 orchards and castor-oil plants at 2 sites along road verges were sampled (Table 1). In Feb 2016, tomato, both wild and cultivated, potato, orange, castor-oil plant, and wild cotton along road verges adjacent to natural populations of *J. clavuligera* bearing *P. longifila* galls were

sampled at 3 sites (Table 1). In addition, *Cnidosculus tubulosus* (Müll. Arg.) I. M. Jonson (Euphorbiaceae) co-existing with *J. clavuligera* in Pulquina Cacti Garden also was sampled to check if the gall midge occurs on other closely related plants. The shoot terminals of tomato, wild cotton, castor-oil plant, and *C. tubulosus* were checked for *P. longifila* larval incidence.

DATA ANALYSIS

Analysis of variance (ANOVA) was used to compare the relative incidence of gall induction by *P. longifila* on *J. clavuligera* in Apr 2013, Nov 2014, Mar 2015, and Feb 2016 with individual sites nested within each season. ANOVA was applied to compare gall incidence at Pulquina Cacti Garden, San Isidro, and La Villa near Punata sites in Feb 2016 with individual plants used as replicates. The means were compared using Tukey's HSD test. Regression analysis was employed to study the relationship between the number of branches per plant in *J. clavuligera* and the percentage of shoot tips with *P. longifila* galls. Statistical analyses were carried out using Sigmastat version 3.5.

Results

GALL MORPHOLOGY

In Bolivia, *P. longifila* induces rosette galls consisting of malformed and abnormally thickened leaves and petioles by modifying axillary and terminal vegetative buds of *J. clavuligera* (Fig. 1A). Developing larvae feed on the nutritive cells that form the inner-most layer in the gall, with no evidence of either cell necrosis or fungal mycelia in them (Fig. 1B). Larval feeding on shoot terminals results in the telescoping of the shoot axis, involving the inhibition of normal shoot growth, in 2 to 3 wk. The thickened and poorly differentiated leaves therefore crowd at shoot terminals. They do not spread out, but curl inwards. With maturation, basal portions of the petioles, rachis, and leaf blades thicken, including hypertrophied cells. The overall color changes from green to purple-red. Due to gall development, the shoot terminals are completely destroyed, often resulting in shoot-tip dieback.

GALL INDUCTION AND DEVELOPMENT ON *JATROPHA CLAVULIGERA*

Bolivian populations of *P. longifila* induce galls on young shoot buds of *J. clavuligera*. Neonate larvae settle in primordial leaf axils of buds and feed at the axillary positions (Fig. 2A). This action induces shoot buds to grow into galls, each consisting of thickened stem axis, leaf blades, and petioles, including manifold layers of parenchyma (Fig. 2B, C). Because of larval feeding, the epidermal cells turn hypertrophied and hyperplasied (Fig. 2D). The epidermal cells close to larval feeding locations divide repeatedly vertically, while those along the sides of the larval feeding areas show fewer vertical divisions, but are immensely enlarged. The cortical parenchyma cells divide and enlarge consequent to larval feeding (Fig. 2E). The outer cortical cells of the stem, and a few of the epidermal cells differentiate into 'nutritive' cells. They bear thin cell walls, large nuclei, and dense cytoplasm (Fig. 2F). As the galls grow with age, they accumulate phenolic materials in their inner cortical cells (Fig. 2G, H).

GALL INCIDENCE ON *JATROPHA CLAVULIGERA*

Gall induction by *P. longifila* was evident in all sites with *J. clavuligera* in Bolivia, resulting in shoot tip dieback. Incidence of *P. longifila* galls (percentage of plants bearing galls) on *J. clavuligera* varied widely
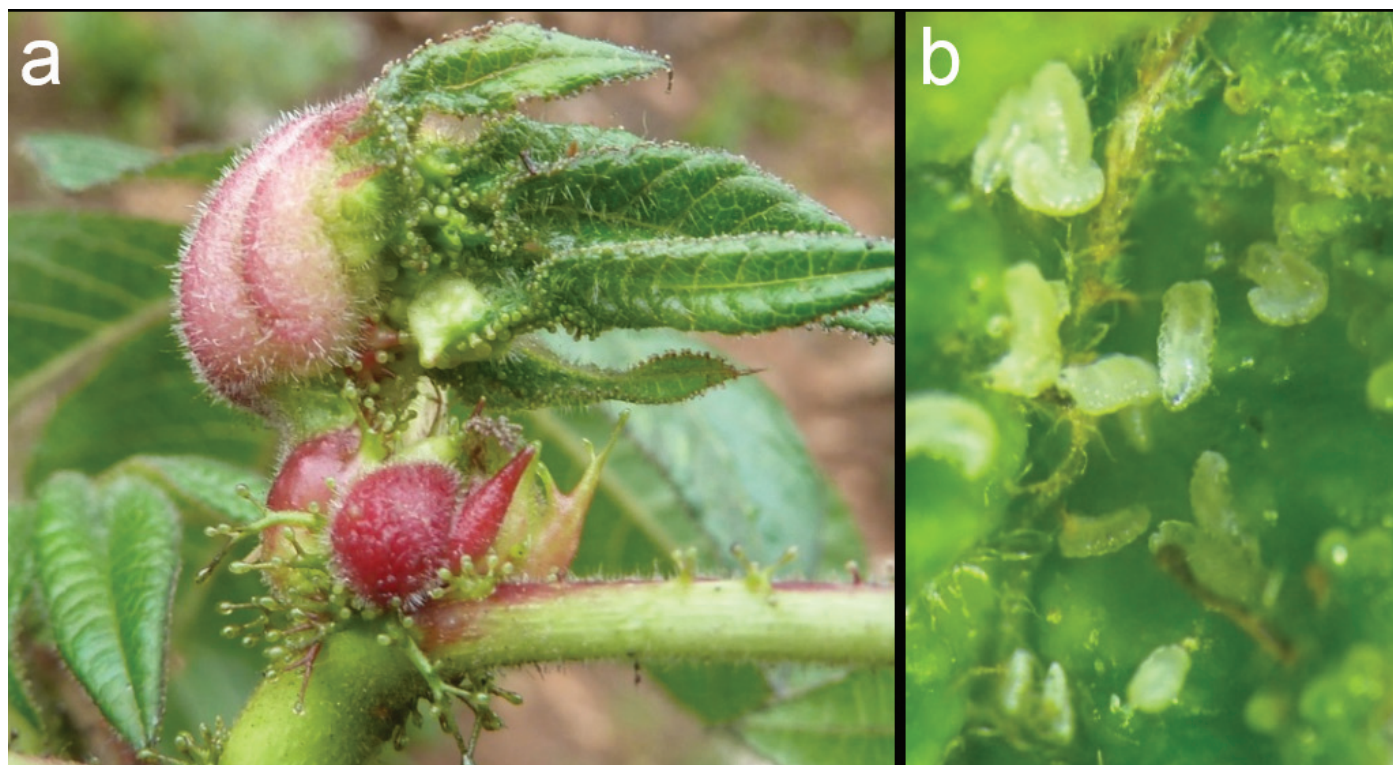
Fig. 1. Rosette galls induced by *Prodiplosis longifila* in the growing shoot tip of *Jatropha clavuligera*. (**A**) Rosette gall in the terminal and axillary buds of *Jatropha clavuligera* with swollen petiole and rachis and malformed leaves. (**B**) Young gall in *Jatropha clavuligera* showing early stage larvae feeding on the nutritive tissue of the gall with no evidence of cell necrosis and fungal growth.

between sites and between yr (Fig. 3A). Although *P. longifila* galls were evident throughout the summer (Nov–Apr; Fig. 3B), live larvae within galls were more abundant in the summer–wet season (Mar 2015, Feb 2016) than in the late dry (Nov 2014) and early dry seasons (Apr 2013) ($F_{3,16}$ = 20.19; P < 0.001; Fig. 3B). In Apr 2013, only 3 of the 113 galls collected had live larvae, whereas none of the 64 galls sampled in Nov 2014 had any live larvae. In contrast, the majority of galls sampled in Mar 2015 (n = 99) and Feb 2016 (n = 264) had live larvae in galls. The number of shoot terminals with galls increased with an increase in the number of branches per plant in all 3 sites (Fig. 4A), and the percentage of shoot tips with galls were significantly higher at La Villa, Punata, than at San Isidro and Pulquina Cacti Garden ($F_{2,26}$ = 11.37; P < 0.001; Fig. 4B). Shoot tips with gall damage usually resulted in the shoot tip dieback causing stunted plant growth, and occasionally plant death.

SUSCEPTIBILITY OF *JATROPHA GOSSYPIIFOLIA* TO GALL INDUCTION BY *PRODIPLOSIS LONGIFILA*

In Bolivia *J. gossypiifolia* and *J. clavuligera* did not co-occur. There was no evidence of *P. longifila* galls on *J. gossypiifolia* populations in home gardens in Santa Cruz and Angostura (Table 1). However, in the no-choice quarantine trials conducted in South Africa, *P. longifila* induced galls on *J. clavuligera* (Fig. 5A) and *J. gossypiifolia* (Fig. 5B) were morphologically similar, as determined through visual observation based on relative gall sizes. In the field transplant experiment conducted in Pulquina Cacti Garden in Bolivia, all of *J. clavuligera* and all of transplanted *J. gossypiifolia* (n = 16) bore old galls (galls with no live larvae of *P. longifila*) with deformed and crinkled leaves (Fig. 5C). Destructive sampling of galls revealed no live larvae on either *J. clavuligera* or on the transplanted *J. gossypiifolia* after 1 yr (in Mar 2015). In Feb 2016 (after 2 yr), only 5 of the 16 transplanted *J. gossypiifolia*

survived, but *P. longifila*-induced galls were evident on all of them, with live larvae. Likewise, *P. longifila* galls with live larvae were evident on all 12 surviving *J. gossypiifolia* seedlings in polybags under shade in the Pulquina Cacti Garden.

PRELIMINARY CHOICE TESTS IN QUARANTINE

In choice tests, although conducted with only a few females and with no repetitions possible, galls developed on both *J. clavuligera* and *J. gossypiifolia*, but not on *J. curcas*, *J. zeyheri*, *R. communis*, *C. gratissimus*, *S. cupulare* (all Euphorbiaceae), nor on tomato, bell pepper and lemon. Evidence of deformation of new growth in shoot tips of both *J. clavuligera* and *J. gossypiifolia* became visible 1 wk after oviposition on them, and normal gall and larval development occurred, as determined through visual observation on relative gall sizes.

FIELD HOST RANGE

There was no evidence of *P. longifila* galls on any other species of *Jatropha* (viz., *J. excisa*, *J. curcas*, *J. hieronymii*, and *J. gossypiifolia*) in Bolivia, although none co-existed with *J. clavuligera* at any sampling site (Table 1). Sampling of *Cnidosculus tubulosus* (Müll. Arg.) I. M. Jonson (Euphorbiaceae), co-existing with *J. clavuligera* in Pulquina Cacti Garden, revealed no galls induced by *P. longifila*.

In the transplant field trial in Bolivia, involving potato, tomato, bell pepper, orange, and castor-oil plant, only the orange plants survived after 1 yr (Feb 2016) while the others died due to drought. Although galls were evident on *J. clavuligera* plants (9.5 ± 7.5 galls per plant) that were used as source-plants for the *P. longifila*, no evidence of *P. longifila* damage on the orange plants occurred, although the lack of young, fresh leaves on the orange plants made the results unreliable. Hence,
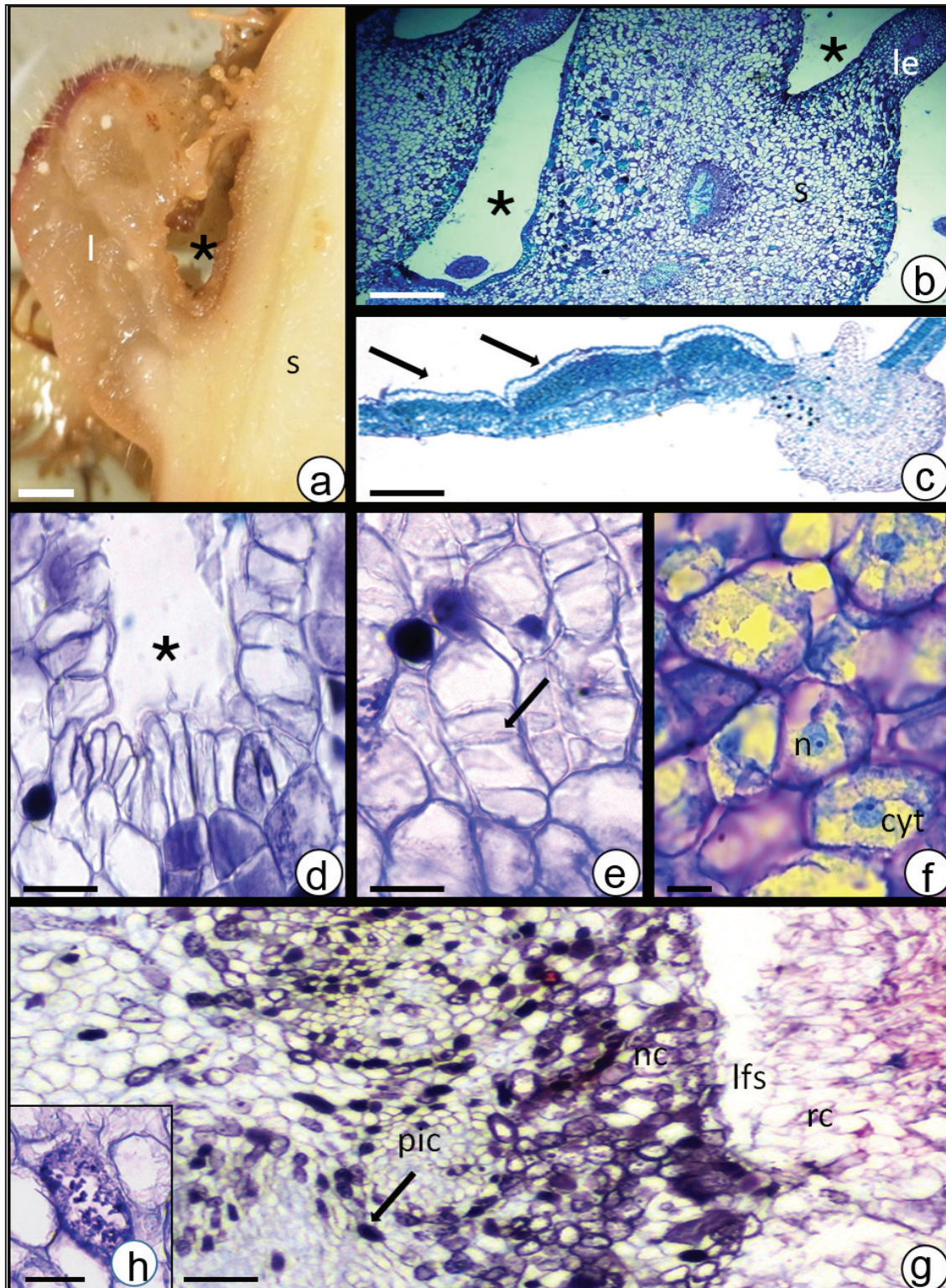
Fig. 2. Morphological and histological changes in the rosette galls induced by *Prodiplosis longifila* on *Jatropha clavuligera*. **(A)** Vertical sectional view of a portion of the galled shoot showing the inflamed axillary position (★) (bar = 1 mm). **(B)** Cross sectional view of a portion of the galled shoot showing the hyperplasied stems and leaves (bar = 0.33 mm); ★ indicates the location where the larvae congregate; le = leaf. **(C)** Cross sectional view of a galled leaf showing the intense hyperplasied upper mesophyll (bar = 0.18 mm); arrows point to the hypertrophied epidermis. **(D)** Cross sectional view of a leaf axil (bar = 0.33 mm); ★ indicates the location where the larvae congregate; note the vertically dividing epidermal cells towards the bottom, whereas the laterally occurring epidermal cells are hypertrophied. **(E)** Cross sectional view of the stem showing variously dividing cortical cells (bar = 30 μm); the arrow indicates cell proliferation. **(F)** Compactly arranged parenchymatous nutritive cells, each with a prominent nucleus (n) and dense cytoplasm (cyt) (bar = 10 μm). **(G)** Low magnification image of galled stem showing layers of nutritive cells (nc), where the larvae feed (lfs) (bar = 30 μm). In fact, the cells lying opposite, which appear to be empty, show signs of regeneration (rc). In the far interior of the stem cortex, numerous phenol-included cells (pic) occur. Far interior into the stem cortex numerous phenol including cells (pic) occur. **(H)** An enlarged view of a phenol-including cell (bar = 18 μm).
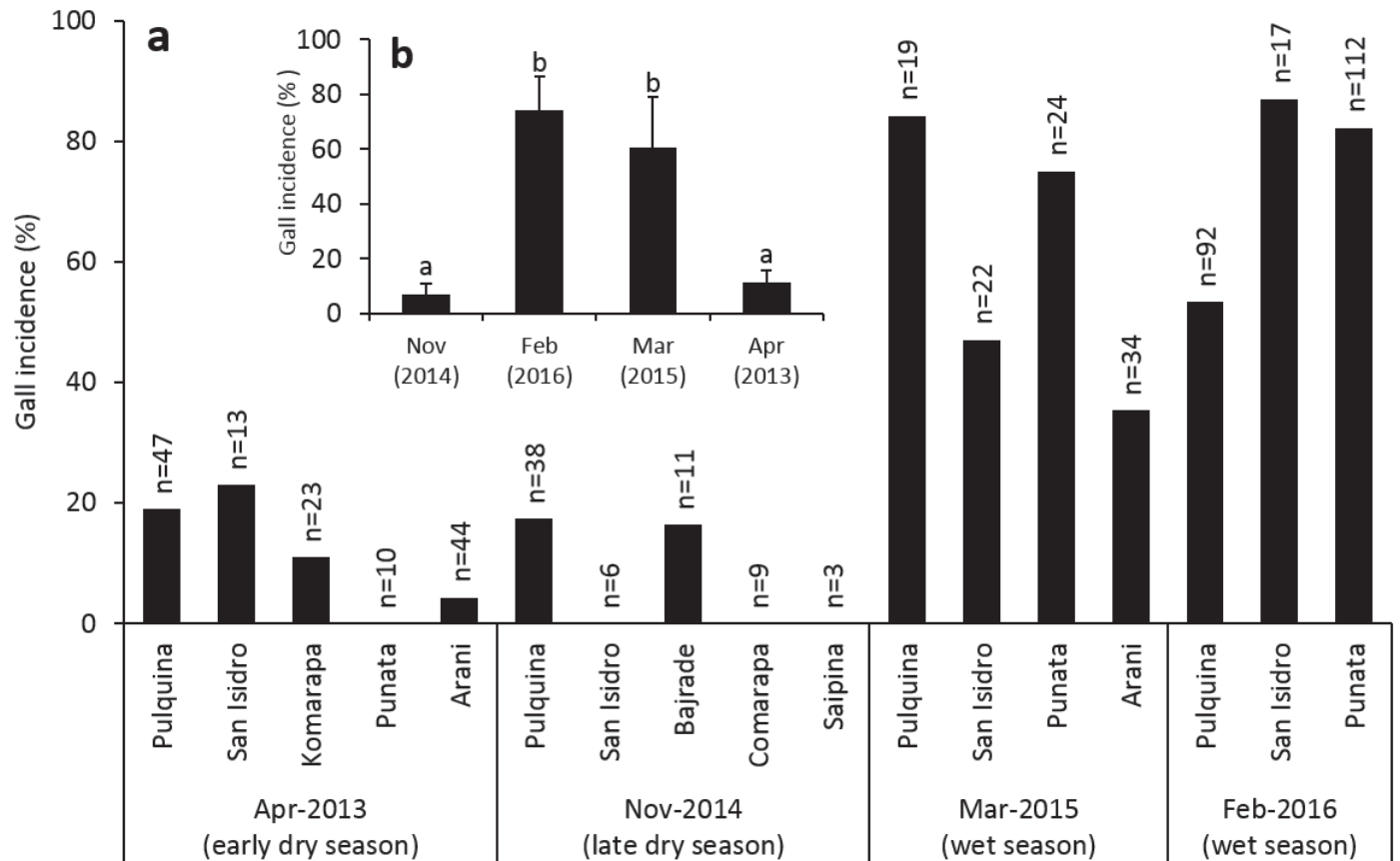
Fig. 3. Incidence of *Prodiplosis longifila* galls on *Jatropha clavuligera* (percentage of plants with galls) in relation to (**A**) sites and (**B**) sampling yr and season (± SE). Different letters above SE bars indicate significant differences (Tukey's test, *P* < 0.001).
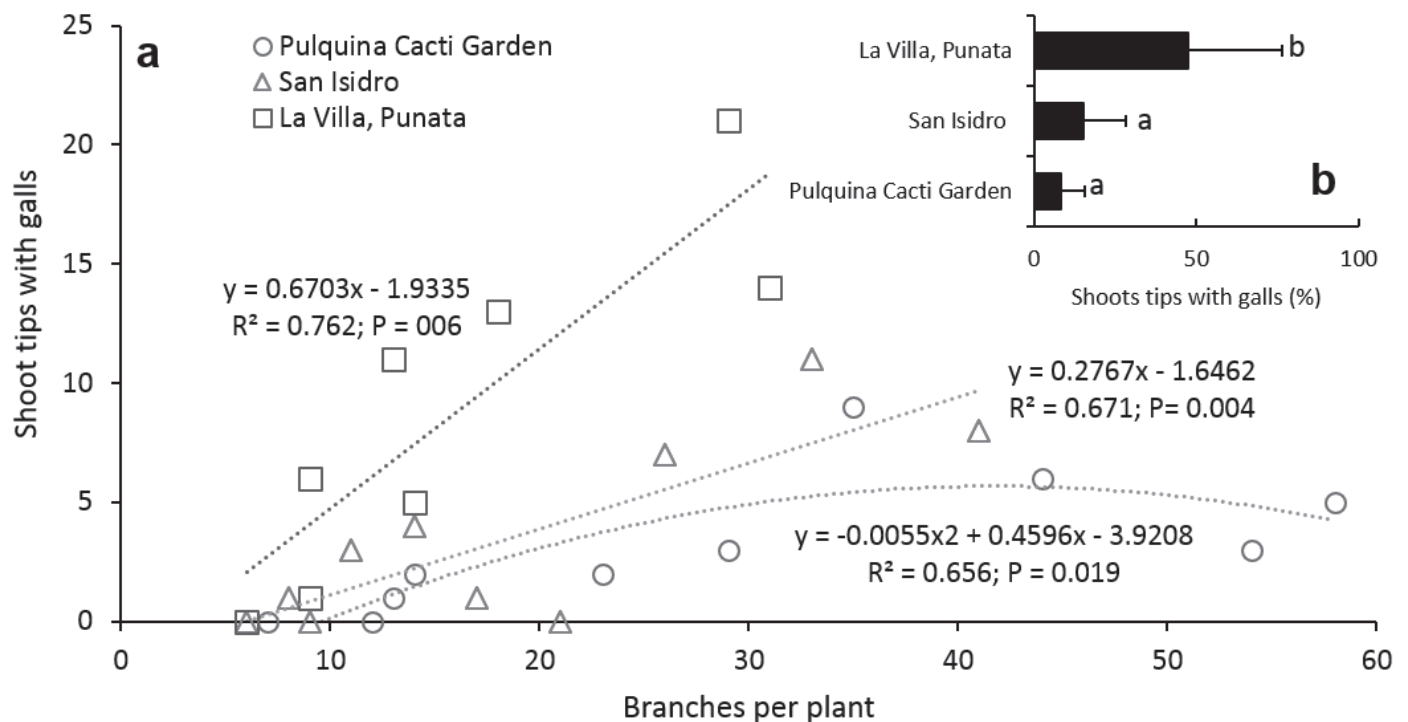
Fig. 4. Relationship between the number of branches per plant and number of shoot tips with *Prodiplosis longifila* galls (**A**) and percentage of shoots (± SE) with *Prodiplosis longifila* galls (**B**) across 3 sites in Bolivia in Feb 2016. Different letters above SE bars indicate significant differences (Tukey's test, *P* < 0.001).
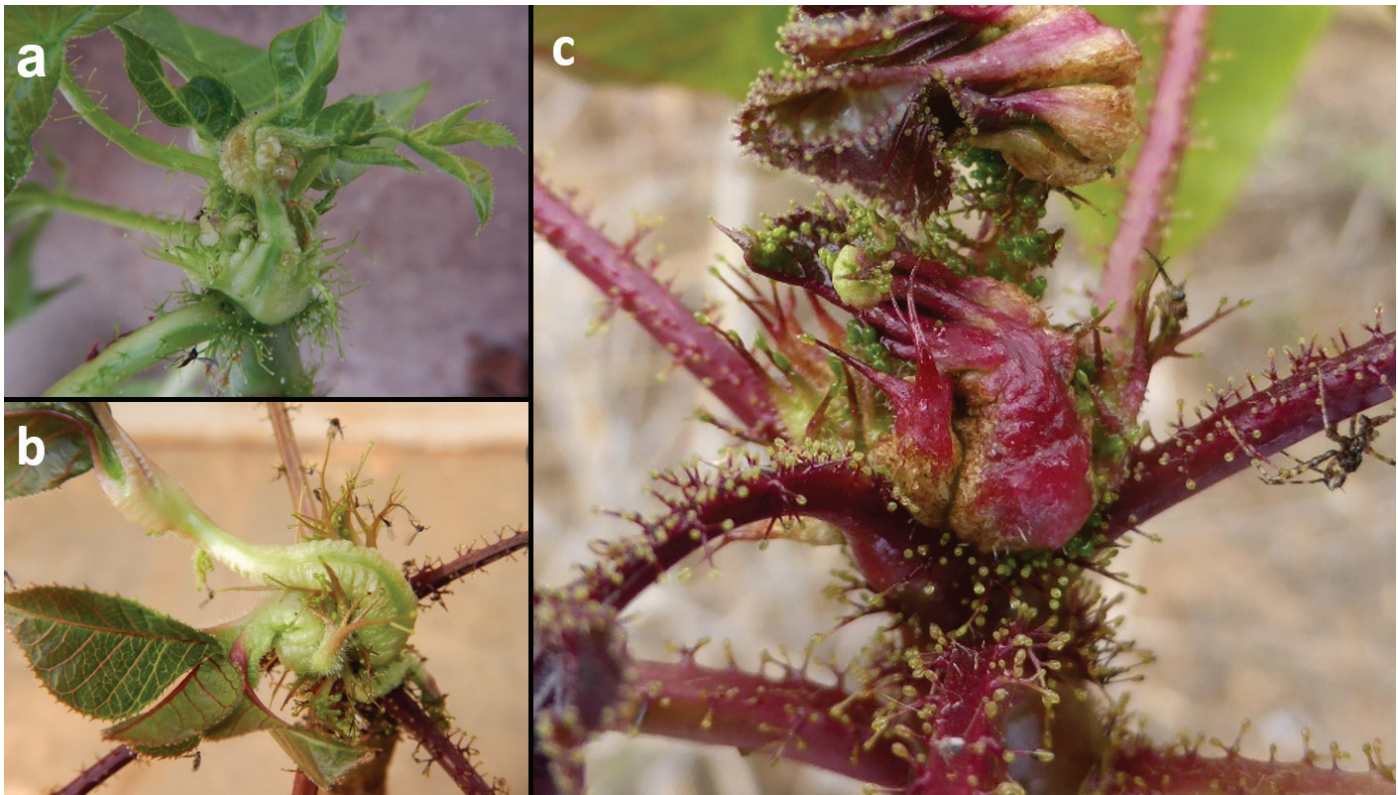
Fig. 5. Susceptibility of *Jatropha gossypiifolia* to *Prodiplosis longifila*. Morphologically similar galls induced on control *Jatropha clavuligera* (**A**) and *Jatropha gossypiifolia* (**B**) plants under no-choice conditions in quarantine in Pretoria, South Africa. Galling on a transplanted *Jatropha gossypiifolia* plant under the natural field conditions in Bolivia (**C**).

we sampled tomato, potato, orange, bell pepper, cotton, and castor-oil plants growing at 5 to 100 m distance from *J. clavuligera* bearing galls.

In spite of a high level of gall incidence on *J. clavuligera* (53–87% of plants with galls), no damage occurred on tomato, potato, orange, bell pepper, and castor-oil plant sampled at Pulquina Cacti Garden and San Isidro near Comarapa in the Santa Cruz region, or at La Villa near Punata in the Cochabamba region (Table 1). Shoot terminals of tomato, orange, and wild cotton plants examined under a stereomicroscope confirmed that no larvae of *P. longifila* occurred on them.

Discussion

Phytophagous Cecidomyiidae generally have narrow host-plant ranges (Gagné 1986) with more than 90% of them inducing galls on a single host-plant species (Skuhrava et al. 1984; Yukawa & Rohfritsch 2005). Therefore, in Cecidomyiidae taxonomy, species distinctions are often made on the basis of host association (Gagné 1986). The Cecidomyiidae that induce galls on *J. clavuligera* in Bolivia have been identified as *P. longifila*, a polyphagous species of the Neotropics. Gall-inducing symptoms of *P. longifila* on *J. clavuligera* appear starkly different from the effects of *P. longifila* reported previously on lime and orange flowers, tomato leaves, flowers and fruits, potato leaf buds, bell pepper and chili pepper leaves and fruits, artichoke flower buds, cucurbit leaves and fruits, bean fruits, leaves and flowers, avocado leaves, grapevine leaves, asparagus spears, and onion leaves in Peru, Ecuador, Florida (USA), and Colombia. No reports of *P. longifila* inducing galls on crops and other hosts from the Neotropics exist (Peña et al. 1989; Anonymous 2013; Hernandez et al. 2015; Maria Manzano, personal communication). Based on gall morphology and field host range, we

propose that the gall-inducing *P. longifila* on *J. clavuligera* in Bolivia is a species with host range restricted to a few *Jatropha* species, and not a polyphagous pest species.

Among gall-inducing Cecidomyiidae, feeding activity of the first instar larvae induces galls that trigger the differentiation of metaplasied cells, which serve as the source of nutrition for the feeding larvae (Rohfritsch 1992). In Bolivia, *P. longifila* larvae induced galls on actively growing shoot terminals of *J. clavuligera* with young leaf primordia. Developing larvae feed on nutritive cells that line the inner cell layers of the gall and, on maturation, the larvae exit galls and pupate in the soil. Being a seasonally deciduous plant, fresh galls appear in the rainy season, when the new flush of leaves appears. Gall induction continues through the wet season (Nov–Mar). Annual sampling suggests that the gall midge remained active during the wet season. In the dry season (Apr–Oct), with no fresh flush of leaves and with all existing leaves shed, there were no fresh galls with larvae. We suspect that with no other known hosts in Bolivia, at the onset of dry season the gall-inducing *P. longifila* overwinter as pupae in the soil, to emerge in the following wet season.

Prodiplosis longifila has been reported as a polyphagous insect feeding on various crops in the Neotropics (https://gd.eppo.int/taxon/PRDI-LO/hosts). So far, there is no record of *P. longifila* as a pest of any crops in Bolivia. This is the first time a gall inducing *P. longifila* has been reported from Bolivia, as well as on any *Jatropha* species. However, gall induction by *P. longifila* was not seen on orange, tomato, potato, bell pepper, or castor-oil plant, known as hosts in other countries, even when growing in the vicinity of *J. clavuligera* with *P. longifila* gall damage. Other phytophagous insects (e.g., Coccoidea on orange, Aphididae and Aleyrodidae on tomato, and Scarabaeidae on potato) were present on the crops, indicating that the absence of *P. longifila* galls or feeding was not due

to pesticide treatments. There was no evidence of *P. longifila* galls on other *Jatropha* species present in the near vicinity (*J. excisa*, *J. curcas*, *J. hieronymii*, and *J. gossypiifolia*), none of which co-occurred with *J. clavuligera*. However, no-choice tests under quarantine conditions in South Africa and susceptibility tests under natural field conditions in Bolivia demonstrated that *J. gossypiifolia* is susceptible and a suitable host for gall induction by *P. longifila*. This suggests that the absence of *P. longifila* galls on *J. gossypiifolia* was likely due to geographic isolation, though it is possibly due to climate incompatibility as well.

Considerable variations occur in the nature of feeding damage, crop plants attacked, and host plants recorded for *P. longifila* in different countries. Although reported as a major pest of tomato, potato, and other horticultural crops in Peru, Colombia, and Ecuador, *P. longifila* has been reported only as a pest of lime and not of tomato or other crops in Florida in the USA. Likewise, *P. longifila* is a key pest of asparagus and potato crops in Peru (Cisneros 1995; Kroschel et al. 2012), but has not been reported as a pest of these crops in Colombia (Caicedo & Bellotti 2001; Hernandez et al. 2015). In Ecuador, *P. longifila* has not been reported on lime, though it is regarded as a pest of lime in Florida (USA), Peru, and Colombia. It has therefore been suggested that *P. longifila* populations collected from different hosts might correspond to a complex of cryptic species rather than a single polyphagous species (EPPO 2015). Based on ecological, behavioral and genetic studies, existence of host-related cryptic species have been identified in other Cecidomyiidae (Lloyd et al. 2005; Dorchin et al. 2009, 2015; Cook et al 2011; Mathur et al. 2012; Fitzpatrick et al. 2013). A similar approach, therefore, is needed to determine if the populations of *P. longifila* collected from different host plants in different countries correspond to a complex of cryptic species (Carneiro et al. 2009; Mathur et al. 2012). Due to difficulties in accessing the biological materials from multiple crops in multiple countries, the easier and quicker approach would be to conduct robust no-choice host-specificity tests in quarantine to resolve whether the gall-inducing *P. longifila* on *J. clavuligera* in Bolivia is a host-specific species with host range restricted to a few *Jatropha* species, or a polyphagous pest species with a wide host range.

In classical weed biological control programs there are several examples of successful exploitation of gall-inducing Cecidomyiidae as effective biological control agents (Winston et al. 2014). There are examples also of successful exploitation of insects sourced from plant species closely related to the target weed species in the native range (Dodd 1940; Julien 2012; Palmer & McFadyen 2012; Palmer & Sims-Chilton 2012; van Klinken & Morin 2012). With no native *Jatropha* species in Australia and with *Jatropha* species not regarded as major ornamental or food crops, a similar strategy of exploiting gall-inducing *P. longifila* sourced from *J. clavuligera* in Bolivia as a "new-association" biological control agent for *J. gossypiifolia*, if proven specific to a few closely related *Jatropha* species, appears promising.

Acknowledgments

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