

# Colonization by Biological Control Agents on Post-Fire Regrowth of Invasive *Lygodium microphyllum* (Lygodiaceae)

Aaron S. David,<sup>1,3,✉</sup> Nicole Sebesta,<sup>2</sup> Anwar A. Abdel-Kader,<sup>1</sup> and Ellen C. Lake<sup>1</sup>

<sup>1</sup>USDA-ARS Invasive Plant Research Laboratory, 3225 College Avenue, Fort Lauderdale, FL 33314, <sup>2</sup>Department of Biological Sciences, Florida International University, Miami, FL 33199, and <sup>3</sup>Corresponding author, e-mail: [aaron.david@usda.gov](mailto:aaron.david@usda.gov)

Subject Editor: Raghu Sathyamurthy

Received 1 April 2020; Editorial decision 3 June 2020

## Abstract

Integration of biological control with other management tactics such as prescribed burning is often important for successful invasive weed control. A critical step in this integration is determining whether the agent can colonize postburn growth of the weed. Here, we investigated postburn colonization by biological control agents on regrowth of the invasive vine *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae, Old World climbing fern) in Florida. We monitored regrowth and subsequent colonization of two agents already established in Florida—the gall-inducing mite *Floracarus perrepae* Knihinicki and Boczek (Acariformes: Eriophyidae) and the foliage-feeding moth *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae)—following three prescribed burns. We provide the first report of natural colonization by the *F. perrepae* mite and *N. conspurcatalis* moth on postburn *L. microphyllum* regrowth, and this colonization typically began 5–9 mo postburn. Furthermore, we report that *L. microphyllum* can recover to prefire levels of percent cover in as little as 5 mo. Our findings indicate that biological control of *L. microphyllum* has the potential to be integrated with prescribed burns.

**Key words:** weed biological control, Old World climbing fern, integrated weed management, eriophyid mites, crambid moths

Management of invasive weeds is one of the foremost challenges for land managers, and integration of different management techniques is often seen as the best course of action for controlling these species (Buhler 2002). Biological control can be used in conjunction with other tactics such as herbicide application, mechanical removal, and prescribed burns (Ainsworth 2003, Hatcher and Melander 2003, Miller 2016). Yet, a recent review reveals that we know surprisingly little about the efficacy of integrating biological control with other techniques, particularly with fire (Lake and Minteer 2018). In some cases, the combination of fire and biological control increases target weed suppression (e.g., DiTomaso et al. 2006, Herrera-Reddy et al. 2012, Tipping et al. 2017), but these strategies can also be incompatible, particularly if the agent responds negatively to fire (Briese 1996, Fellows and Newtown 1999, Zouhar et al. 2008). Given that many invasive plants occur in fire-managed habitats (Brooks et al. 2004), it is critical to understand the interactions between biological control agents and fire in order to advance management goals.

One way in which biological control can effectively integrate with fire management is via rapid establishment of agents on the recovering weed following a prescribed burn. Weeds vary in their types of recovery responses to fire (e.g., resprouting from rhizome or trunk, seedling recruitment, dispersal into burned areas)

and, therefore, may vary in their recovery times (Pyke et al. 2010). Biological control agents that quickly establish on new growth may inflict severe damage on the developing plant. For example, attack rates by *Eustenopus villosus* on yellow starthistle, *Centaurea solstitialis*, in California were highest 1 yr postburn (DiTomaso et al. 2006), and the populations of *Oxyops vitiosa* on *Melaleuca quinquenervia* were unaffected by fire and rapidly colonized postfire (Tipping et al. 2017). However, such data on postfire colonization dynamics of biological control agents is generally lacking (Lake and Minteer 2018). As such, a clearer understanding of the interplay between invasive weed recovery and biological control agent establishment on regrowth will contribute to the development of integrated weed management plans.

*Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae, Old World climbing fern) invades a suite of fire-adapted habitats in Florida, including pine flatwoods and sawgrass prairies, as well as other common habitats such as cypress swamps and Everglades tree islands (Pemberton and Ferriter 1998, Hutchinson et al. 2006). This invasive vine, which is native to parts of tropical and subtropical Australia, Asia, and Africa, was first reported as naturalized in Florida in 1965 (Beckner 1968, Pemberton 1998) and is considered one of the most noxious invasive weeds in Florida (FLEPPC 2019). *Lygodium*

*microphyllum* produces multiple, indeterminately growing rachises (individual leaves or fronds) from a rhizome located at or near the soil surface (Mueller 1982). These rachises can reach 30 m in length, smothering native vegetation while trailing horizontally or climbing vertically and forming 'tree skirts' (Pemberton 1998, Hutchinson et al. 2006). Trailing *L. microphyllum* can form 1 m thick rachis mats that decrease the abundance of native flora (Brandt and Black 2001, Hutchinson et al. 2006). Prescribed burns of *L. microphyllum* are useful for removing dead rachises, but rachises within tree skirts can negatively alter fire behavior by acting as a ladder that carries fire into the canopy and damages tree crowns (Hutchinson et al. 2006 and references therein). The plant can survive and resprout from the rhizome following fire, sometimes within weeks (N.S., personal observations), and infestations can return to preburn levels within a year (Hutchinson et al. 2006, Stocker et al. 2008).

Two biological control agents introduced to help manage *L. microphyllum* have established in Florida (Boughton and Pemberton 2009, Boughton and Pemberton 2011, Lake et al. 2014)—the gall-inducing mite *Floracarus perrepae* Knihinicki and Boczek (Acariformes: Eriophyidae) and the foliage-feeding moth *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae). *Floracarus perrepae* galls at the margins of subpinnae (subbleaflets) can lead to leaf necrosis (Goolsby et al. 2004), and mites can attack the growing rachis tips and subsequently reduce climbing ability (David and Lake 2020). *Neomusotima conspurcatalis* feeds on the subpinnae in its larval stage, and causes massive defoliation of large areas of *L. microphyllum* during outbreaks (Boughton and Pemberton 2009). Field releases of both agents began in 2008 and resulted in initial establishment (Boughton and Pemberton 2009, 2011; Lake et al. 2014). Since 2014, both agents have been mass-reared and released by the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Ft. Lauderdale, FL (26°5'6"N, 80°14'24"W), resulting in widespread establishment throughout much of the *L. microphyllum* range in south and central Florida. Because both agents complete their development entirely within the *L. microphyllum* foliage and are multivoltine, colonization of postburn *L. microphyllum* regrowth would be likely attributable to individuals dispersing from nearby infestations or unburned plants.

Here, we investigated the colonization by these two biological control agents on regrowth of *L. microphyllum* following prescribed burns. We monitored regrowth of surviving *L. microphyllum* and subsequent colonization by naturally dispersing populations of *F. perrepae* and *N. conspurcatalis* following prescribed burns at three sites in south and central Florida—Everglades National Park, the Arthur R. Marshall Loxahatchee National Wildlife Refuge, and Archbold Biological Station.

## Materials and Methods

We chose sites across a range of habitats managed with fire. At each site, we established monitoring locations to measure *L. microphyllum* regrowth and biological control agent colonization. Because sites varied widely (e.g., extent of the *L. microphyllum* infestation, severity of the burn), we tailored our monitoring designs to each site.

### Everglades National Park

Two prescribed burns were conducted in 2017 at Everglades National Park in Cape Sable, FL. Both burns occurred within a single treated site (25.219°N, -81.072°W) with a sparse infestation of *L. microphyllum*, which facilitated the selection of a

total of 184 individual plants across three sampling locations for monitoring. In pretreatment sampling (January 2017), these plants averaged 0.125 m<sup>2</sup> in cover and 1 m in height. The first burn was conducted in February 2017, and thoroughly burned two of the three sampling locations. Because these locations experienced the same prescribed burn, we pooled the data from these two locations ( $n = 132$  surviving plants), and collectively refer to them as 'Location 1.' The second burn was conducted in August 2017, and effectively burned—though patchily—the remaining location ('Location 2,'  $n = 51$  surviving plants) without affecting Location 1 plants. For both locations, we only included those selected plants whose aboveground tissues were completely consumed by the fire in our analyses. Plants were surveyed in January 2017 prior to the first burn in May 2017 (3 mo postburn for Location 1, 3 mo preburn for Location 2), and then at 6 mo intervals through May 2019 (27 and 21 mo postburn, respectively). During each sampling period, we recorded the presence or absence of *F. perrepae* and *N. conspurcatalis* on the selected plants. Beginning in May 2017, amount of damage was quantified by visually estimating the percentage of each plant's aboveground tissue with agent damage according to the following scale: 1 = 0.01–10%; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–90%; 6 = 91–100%. *Floracarus perrepae* damage was quantified based on galls found at the margins of subpinnae, and *N. conspurcatalis* damage was based on unique 'windowing' foliar damage to subpinnae and the presence of frass. During the May 2017 pretreatment survey of Location 2, we added 1 additional plant to the survey.

We evaluated whether agent activity at each sampling period differed from the initial pretreatment measurement. For each prescribed burn, we fit a mixed-effects generalized linear model with binomial error that used a random effect of the selected plant to account for repeated measures. Percent damage was converted to a continuous variable by taking the midpoint of each category. However, due to low variability in percent damage (most damage estimates were scored as 0 or 1), and complete absence of *N. conspurcatalis* at the site (see Results), we instead analyzed presence/absence of only *F. perrepae*. The models fit the presence of the agent as a function of the sampling period, and we used contrasts with the January 2017 pretreatment data to determine significant changes in gall presence. All data were analyzed with R v. 3.6.1 (R Foundation 2019) and the *lme4* package (Bates et al. 2015).

### Arthur R. Marshall Loxahatchee National Wildlife Refuge

A prescribed burn on a large strand island within the Arthur R. Marshall Loxahatchee National Wildlife Refuge (hereafter Loxahatchee NWR; 26.458° N, -80.257° W) was conducted in May 2019. The strand island was partially inundated for much of the year. Both *F. perrepae* and *N. conspurcatalis* have established on *L. microphyllum* infested tree islands and cypress swamps within Loxahatchee NWR (A.S.D. and E.C.L., unpublished data), suggesting that the strand island could be colonized by nearby, dispersing agent populations following the prescribed burn.

In April 2019, prior to the burn, we set up 24 0.5 × 0.25 m plots to survey for *F. perrepae* and *N. conspurcatalis*. Unlike the relatively sparse infestations in the Everglades study, the infestations at Loxahatchee NWR and Archbold Biological Station (described subsequently) were too dense to distinguish individual plants, as is often the case in *L. microphyllum* infestations (A.S.D and E.C.L., personal observations); therefore, we used plots as the sampling unit for these latter two studies. The prescribed burn was patchy, and, following

the burn, we first evaluated whether each plot was burned or unburned. Next, we attempted to pair the original plots with new plots of the opposite burn status (1–5 m apart). We were able to pair 16 of the original plots with 16 new plots for a total of 32 plots (16 pairs of burned/unburned plots). We visually estimated *L. microphyllum* percent cover and damage caused by biological control agents using a scale similar to that described earlier—1 = 0–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%—but using plots instead of individual plants in June 2019 (1 mo posttreatment), September 2019 (4 mo), and January 2020 (8 mo). For each sampling period, we analyzed differences in *L. microphyllum* percent cover using paired *t*-tests for each pair of plots. Due to low occurrences of *N. conspurcatalis* and the absence of *F. perrepae* at the site (see Results), we analyzed only the presence of *N. conspurcatalis* by constructing generalized mixed-effects models with binomial error using a plot's burn status as a fixed effect and the plot pair as a random effect. We tested for significance of these models using analysis of deviance using the car package (Fox and Weisberg 2019) in R.

### Archbold Biological Station

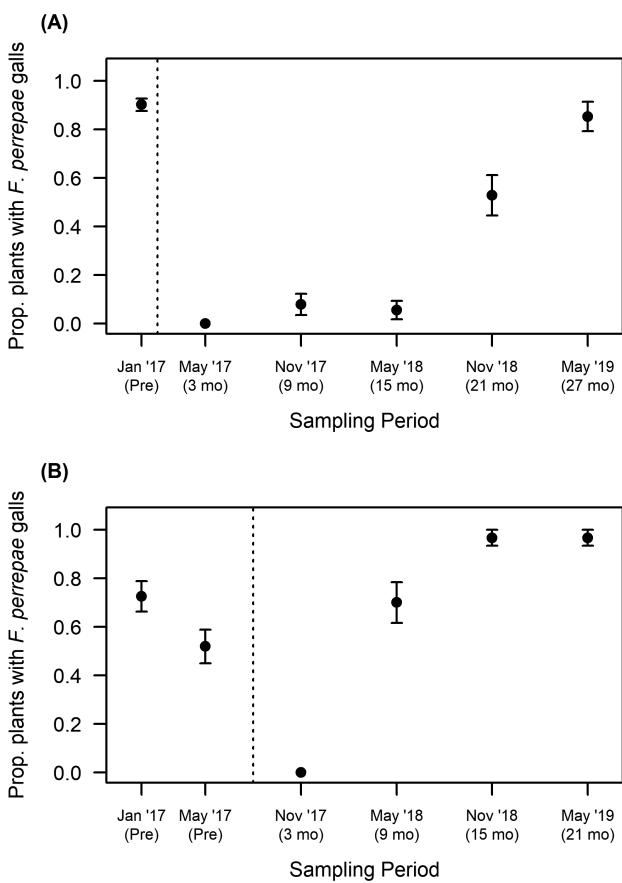
A prescribed burn of a mesic flatwoods habitat (27.131°N, -81.373°W) that had been converted to semi-improved pasture at Archbold Biological Station was conducted in May 2019. We were unable to collect pretreatment data prior to the burn, but previous surveys elsewhere at Archbold and nearby locations indicated that both agents were established in the area (A.S.D. and E.C.L., unpublished data). Therefore, we selected a similar, nearby site to serve as an unburned reference site (27.124°N, -81.382°W). Two months postburn, we set up 12 0.5 × 0.25 m plots as above at both sites. As stated earlier, we recorded *L. microphyllum* percent cover and estimated damage of both biological control agents. Data were collected in July 2019 (2 mo posttreatment), October 2019 (5 mo), and January 2020 (8 mo) at both sites. We analyzed differences in *L. microphyllum* percent cover at each sampling period using *t*-tests in R. Due to low occurrences of *N. conspurcatalis* and the absence of *F. perrepae* in the burned site (see Results), we analyzed only the presence of *N. conspurcatalis* by constructing generalized linear models with binomial error using the site as a predictor variable. We tested for significance of these models using analysis of deviance as stated earlier.

## Results

### Everglades National Park

At Location 1, pretreatment sampling in January 2017 indicated that 90% of selected plants ( $n = 132$ ) had *F. perrepae* galling (Fig. 1A). In total, 48 plants (36%) survived the February 2017 burn and resprouted from rhizomes by May 2017, and we observed continued mortality throughout the study (34 plants remained at 27 mo). Presence of mite galling was significantly lower (8% or fewer of plants had galls) for the first 15 mo posttreatment compared with the pretreatment sample before but, by 21 mo posttreatment, galling was documented on more than 50% of the plants, and neared pretreatment levels by 27 mo posttreatment (85%) (Fig. 1A; Table 1). Average *Floracarus perrepae* damage was generally low (<4%) for all sampling periods but increased in the final sampling period in May 2019 (8%) with the most damaged plant scored as a 3 (26–50% damage). We did not observe *N. conspurcatalis* at any sampling point.

At Location 2, 73% of plants ( $n = 51$ ) had galls in January 2017 in the first pretreatment sampling, but this percentage dropped



**Fig. 1.** Presence of *Floracarus perrepae* mite galls on tagged *Lygodium microphyllum* plants within the treated site at Everglades National Park. (A) Location 1 burned February 2017 (Pretreatment  $n = 132$ , posttreatment  $n$  ranged from 34 to 48). (B) Location 2 burned August 2017 (Pretreatment  $n = 52$ , posttreatment  $n$  ranged from 30 to 40). Points show mean presence of galls on selected plants  $\pm$  1 binomial standard error. Dashed, vertical lines indicate the timing of each burn.

significantly to 52% by May 2017 ( $n = 52$ ) in the second pretreatment sampling (Fig. 1B; Table 1). Following the August 2017 burn, 40 (77%) of the selected plants survived to the next survey in November 2017, and additional mortality continued throughout the study (30 plants remained at 21 mo). Presence of galls on the surviving plants dropped to 0% in November 2017 but rebounded to above pretreatment levels by 9 mo posttreatment, with 70% of the 30 surviving plants galled. By November 2018, nearly all surviving plants were galled (97%) and this remained true at the final survey at 21 mo (May 2019), surpassing pretreatment levels (Fig. 1B; Table 1). *F. perrepae* damage was generally low (<6%) for all sampling periods (including the pretreatment May 2017), though, similar to Location 1, damage increased in the final sampling period in May 2019 (16%) with three plants scored as a 3 (26–50% damage) and 1 plant scored as a 4 (51–75% damage). We did not observe *N. conspurcatalis* at any sampling point.

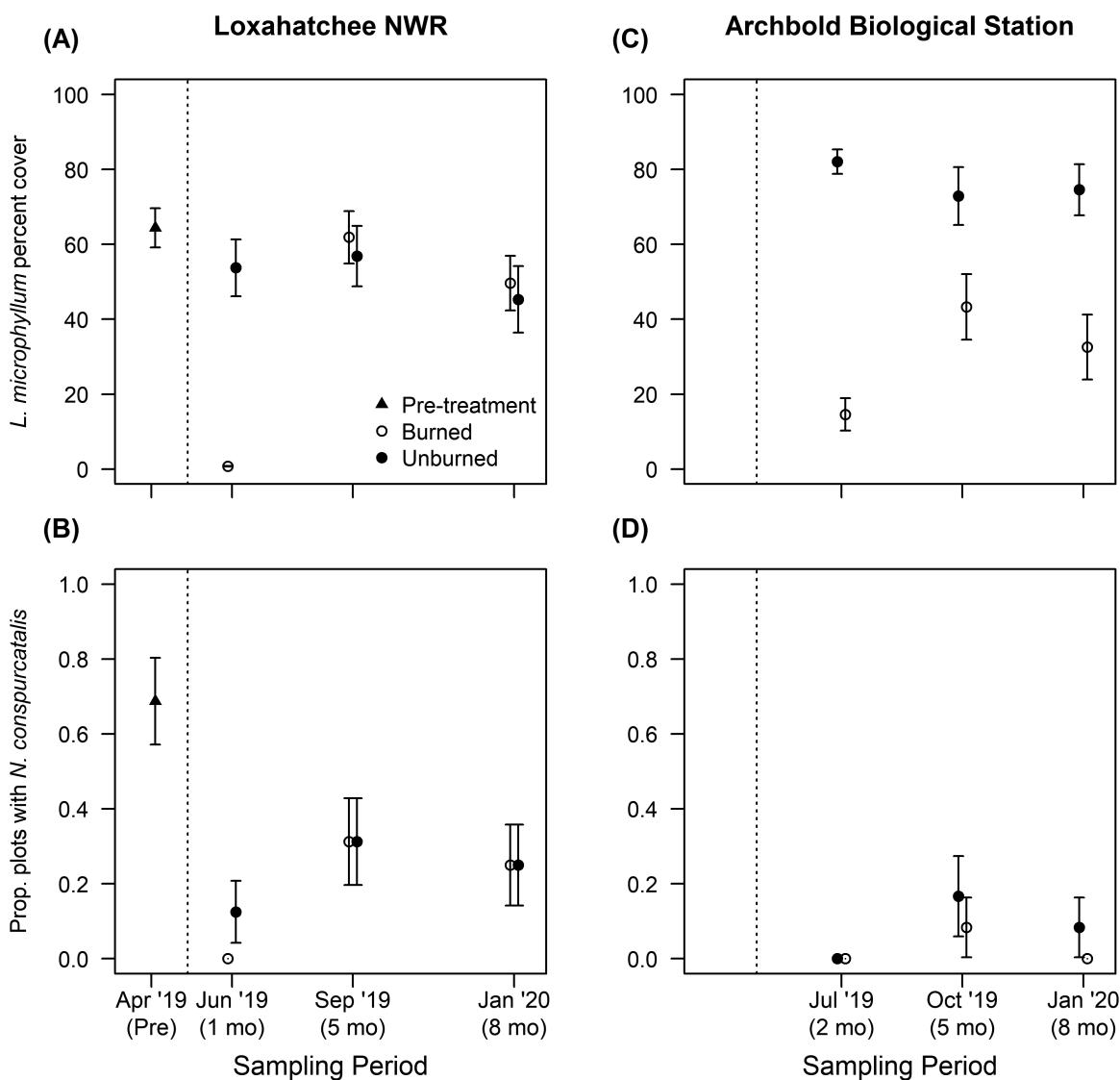
### Loxahatchee NWR

*Lygodium microphyllum* percent cover was higher in unburned plots 1 mo postburn, but burned and unburned plots did not differ in percent cover at 5 or 8 mo posttreatment (Fig. 2A; Table 2). We did not observe *F. perrepae* galling at the site at any sampling period. The presence of *N. conspurcatalis* was widespread and occurred in 69%

**Table 1.** Regression coefficients of the presence of galls on selected plants from two burns at Everglades National Park

	Location 1 (Burned Mar. 2017)				Location 2 (Burned Aug. 2017)			
	Estimate	SE	z	P	Estimate	SE	z	P
(Intercept)	3.15	0.64	4.96	<0.001	1.17	0.39	2.993	0.003
May 2017	-22.72	150.98	—	—	-1.08	0.48	-2.261	0.024
Nov 2017	-6.51	1.30	-4.991	<0.001	-20.79	166.11	—	—
May 2018	-6.99	1.45	-4.815	<0.001	-0.28	0.57	-0.498	0.619
Nov 2018	-2.79	0.67	-4.158	<0.001	2.46	1.10	2.246	0.025
May 2019	-0.44	0.68	-0.652	0.515	2.46	1.10	2.246	0.025

Models were fit using mixed-effects generalized linear models with binomial errors. Comparisons are between the presence of galls at each sampling date and the first pretreatment measurements collected in Jan. 2017. Note that on some sample dates none of the plants had galls present, and due to this lack of variation the statistical parameters could not be interpreted. Bolded P-values indicate significance ( $P < 0.05$ )



**Fig. 2.** Response of *Lygodium microphyllum* and *Neomusotima conspurcatalis* at (A and B) Loxahatchee National Wildlife Refuge and (C and D) Archbold Biological Station. In (A) and (B) adjacent burned and unburned plots were paired. In (C) and (D) the treated burn site was paired with a nearby untreated control site, and no pretreatment data were available. In (A) and (C), points show mean cover  $\pm 1$  standard error. In (B) and (D), points show mean presence  $\pm 1$  binomial standard error.

of plots during pretreatment surveys, but reduced to 6% of plots immediately following the burn (Fig. 2B). Although we detected seasonal variation of *N. conspurcatalis* presence, feeding damage did

not differ between burned and unburned plots at any of the three sampling periods (Table 2). When present, *N. conspurcatalis* damage was generally low (<5%).

**Table 2.** *Lygodium microphyllum* cover and *Neomusotima conspurcatalis* presence between burned and unburned plots at three postburn sample dates at the Loxahatchee NWR and Archbold Biological Station

	<i>L. microphyllum</i> cover			<i>N. conspurcatalis</i> presence		
	<i>t</i>	df	<i>P</i>	$\chi^2$	df	<i>P</i>
Loxahatchee NWR						
June 2019 (1 mo)	7.03	15	<0.001	0.01	1	0.919
Sept. 2019 (5 mo)	0.43	15	0.674	0.00	1	1.000
Jan. 2020 (8 mo)	0.42	15	0.678	0.00	1	1.000
Archbold Biological Station						
July 2019 (1 mo)	12.50	22	<0.001	0.00	1	1.000
Oct. 2019 (5 mo)	2.53	22	0.019	0.39	1	0.534
Jan. 2020 (8 mo)	3.81	22	<0.001	1.43	1	0.232

For Loxahatchee NWR data, burned and unburned plots were paired, and thus analyzed with a paired *t*-test (*L. microphyllum* cover) and an analysis of deviance of a generalized mixed-effects model with binomial error using the random effect of plot pair (*N. conspurcatalis* presence). For Archbold Biological Station data, plots were not paired and were, therefore, analyzed with a *t*-test and an analysis of deviance of a generalized linear model with binomial error, respectively

### Archbold Biological Station

*Lygodium microphyllum* percent cover was consistently lower at the burn site compared to the unburned reference site at each sampling period (Fig. 2C; Table 2). We did not observe *F. perrepae* galling at the burned site, but at the unburned reference site we observed galls in 17, 42, and 58% of plots at the three sampling periods, respectively, indicating the presence of an active *F. perrepae* population nearby. *Neomusotima conspurcatalis* damage was generally low (<5% of *L. microphyllum* in plot damaged when present) and did not significantly differ between the burned and unburned reference sites (Fig. 2D; Table 2).

### Discussion

Establishment of biological control agents on plant regrowth following other management actions is essential for successful integrated weed management. In this study, we provide the first report of colonization by the *F. perrepae* mite and the *N. conspurcatalis* moth on postburn *L. microphyllum* regrowth. *Floracarus perrepae*, which was previously established in some areas of Everglades National Park, reestablished on *L. microphyllum* regrowth following fire, and, to a lesser extent, *N. conspurcatalis* reestablished on *L. microphyllum* regrowth in Loxahatchee NWR and colonized regrowth at Archbold. Furthermore, we report *L. microphyllum* can attain preburn levels of cover within 5 mo postburn, the fastest reported recovery of this invasive vine.

Postburn colonization by weed biological control agents is an important part of successful integrated management (DiTomaso et al. 2006, Herrera-Reddy et al. 2012). We demonstrate here that colonization can occur on relatively short timescales—9 mo for *F. perrepae* and 5 mo for *N. conspurcatalis*. Agent colonization occurred without the assistance of additional releases, and was likely due to dispersal from nearby *L. microphyllum* infestations functioning as source populations. Both *N. conspurcatalis* and *F. perrepae* are capable of long-distance dispersal and have spread across the state beyond their release locations (Lake et al. 2014; A.S.D. and E.C.L., unpublished data). However, agents do not always colonize post-management regrowth and reach damaging population levels quickly enough to limit the impact of the target weed (Paynter et al. 2012). Other limitations to agent colonization, particularly for *F. perrepae*, include potential incompatibility with plant genotype (Goolsby et al. 2006, Boughton and Pemberton 2011) and bottom-up effects (e.g., nutrient availability or inundation) that stress the plant (A.S.D. and

E.C.L., unpublished data). These factors could have contributed to preventing *F. perrepae* colonization at either the Archbold burn site where the sandy soil is especially dry or at the Loxahatchee NWR where the site is inundated for much of the year. Future work should assess whether this natural colonization occurs fast enough for the agents to limit regrowth of *L. microphyllum* or if supplemental releases are needed to achieve desired damage levels.

Changes to the treated site and to plant tissues themselves in response to fire damage can alter colonization of regrowth by herbivores, including biological control agents. For example, studies have documented increases in plant nutritional quality (Vieira et al. 1996) and chemical changes in the leaves of regrowth (Wheeler and Ordung 2006) which could either promote or hinder biological control. Increased management success could result if the combination of biological control and fire increases the susceptibility of the target weed to the agent (Paynter and Flanagan 2004) or the postburn mortality of the weed (Drus et al. 2014, Tipping et al. 2017). In our study, we found that *F. perrepae* did attain higher presence than the pretreatment measurement in one site (Everglades Location 2), though this effect was not observed until 9 mo posttreatment. However, given sufficiently high propagule pressure, it may be possible for *F. perrepae* to preferentially attack regrowth much earlier, and this may be an area for further research.

The presence of the biological control agents in this study could be partly attributable to seasonal dynamics. For example, in a shade house study of within-gall *F. perrepae* activity, populations peaked in late-Spring and mid-Fall (David et al. 2019), and ongoing field monitoring shows widely varying seasonal dynamics of *N. conspurcatalis* both within and among populations (A.S.D. and E.C.L., unpublished data). Indeed, the two pretreatment samples at the Everglades Location 2 site showed a slight drop in the proportion of plants galled between January and May that could have indicated that the mite population had finished peaking at that site. Furthermore, these seasonal dynamics could have interacted with the timing of the burns. At the Everglades site, Location 1 burned thoroughly in the dry season and took longer to regain *F. perrepae*, while Location 2 experienced a relatively patchier burn during the wet season and experienced much faster *F. perrepae* colonization. Patchy burns can result in the creation of refugia, which can act as a source of dispersing agents to regrowth or undamaged plants postburn (Briese 1996). Therefore, it is possible that *F. perrepae* individuals survived on unburned *L. microphyllum* plants in Location 2, and that these plants

acted as a refuge and source for agent reestablishment. Nonetheless, our study still demonstrates that postburn colonization can occur naturally. Future work should be directed towards coordination of postburn *L. microphyllum* regrowth, agent reestablishment, and agent seasonal population dynamics to maximize agent impact on regrowth.

To our knowledge, our study has documented the fastest *L. microphyllum* recovery to preburn levels following fire recorded at 5 mo. Previous work that measured *L. microphyllum* cover on a yearly basis had shown *L. microphyllum* recovery 1 yr postburn (Stocker et al. 2008), and our current findings at Loxahatchee NWR reveal recovery can occur in less than half that time. However, it can be difficult to compare weed responses from one burn to another given that fires vary across habitats, seasons, and fire intensities (Briese 1996, Brooks et al. 2004, Zouhar et al. 2008, Drus et al. 2014). Such comparisons are particularly difficult with field populations of *L. microphyllum* given that we cannot measure an individual plant's belowground biomass resources needed for resprouting. At Loxahatchee NWR, the burn site was partially inundated for at least half of the year; this may have limited the intensity of the burn (Slocum et al. 2003) and the impact on *L. microphyllum* rhizomes at this site, leading to rapid regrowth. Yet, as our Everglades results confirmed, fire can still harm or even kill *L. microphyllum*, particularly if the plant is small or the rhizome is damaged, and can reduce reproductive output through decreased spore production (via destruction of fertile fronds) and spore viability (Hutchinson et al. 2006, Sebesta et al. 2016). Fire is often not an optimal *L. microphyllum* management strategy on its own, but in some cases, may be helpful for removing dead rachis mats, killing small plants, and facilitating recruitment of native plant species (Hutchinson et al. 2006).

Finally, our findings suggest that biological control of *L. microphyllum* has the potential to be integrated into fire management. Studies on such integration in weed control are generally lacking (Lake and Minteer 2018) yet are especially critical given the importance of fire as a management tool in some natural systems. If agents can naturally colonize the regrowth following a burn, this may limit the ability of the plant to regenerate (Tipping et al. 2017). Though large *L. microphyllum* tree skirts currently act as a ladder to carry fire into the canopy and damage tree crowns (Hutchinson et al. 2006), successful biological control agent establishment and impact could slow the growth of rachises into the tree canopy (David and Lake 2020). High populations of *F. perrepae* that limit the growth of tree skirts could eventually facilitate expanded use of fire as a management practice without the risk of damage to vulnerable tree canopies. However, further research is needed to properly evaluate the integration of fire and biological control towards managing *L. microphyllum*. Our demonstration of the ability of agents to establish postburn is an important first step towards integrated management of biological control and fire.

## Acknowledgments

We thank J. Richards, J. Taylor, H. Cooley, and A. Carmona Cortes for discussion and assistance with data collection. We also thank L. Rodgers, R. Gibble, M. Juntunen, E. Allen, and S. Gonzalez for assistance and support at Loxahatchee National Wildlife Refuge, K. Main for support at Archbold Biological Station, Everglades National Park, and ENP Fire and Aviation crews for helicopter transport and facilitation of fieldwork, and Editor R. Sathyamurthy and two anonymous reviewers for their constructive feedback on the manuscript. Everglades National Park retains ownership

of data collected on site, and collection was funded by U.S. National Parks Service Task Agreement number P16AC01505, under Master Cooperative Agreement P16AC00032. Funding was also provided by the U.S. Department of Agriculture (USDA), and the South Florida Water Management District and U.S. Army Corps of Engineers as part of the Comprehensive Everglades Restoration Plan. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity employer and provider.

## References Cited

Ainsworth, N. 2003. Integration of herbicides with arthropod biocontrol agents for weed control. *Biocontrol Sci. Technol.* 13: 547–570.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1–51.

Beckner, J. 1968. *Lygodium microphyllum*, another fern escaped in Florida. *Am. Fern J.* 58: 93.

Boughton, A. J., and R. W. Pemberton. 2009. Establishment of an imported natural enemy, *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) against an invasive weed, old world climbing fern, *Lygodium microphyllum*, in Florida. *Biocontrol Sci. Technol.* 19: 769–772.

Boughton, A. J., and R. W. Pemberton. 2011. Limited field establishment of a weed biocontrol agent, *Floracarus perrepae* (Acariformes: Eriophyidae), against Old World climbing fern in Florida—a possible role of mite resistant plant genotypes. *Environ. Entomol.* 40: 1448–1457.

Brandt, L. A., and D. W. Black. 2001. Impacts of the introduced fern, *Lygodium microphyllum*, on the native vegetation of tree islands in the Arthur R. Marshall Loxahatchee national wildlife refuge. *Florida Sci.* 64: 191–196.

Briese, D. 1996. Biological control of weeds and fire management in protected natural areas: are they compatible strategies? *Biol. Conserv.* 77: 135–141.

Brooks, M. L., C. M. D'Antonio, D. M. Richardson, J. B. Grace, J. E. Keeley, J. M. DiTomaso, R. J. Hobbs, M. Pellatt, and D. Pyke. 2004. Effects of invasive alien plants on fire regimes. *Bioscience*. 54: 677.

Buhler, D. D. 2002. Challenges and opportunities for integrated weed management. *Weed Sci.* 50: 273–280.

David, A. S., and E. C. Lake. 2020. Eriophyid mite *Floracarus perrepae* reduces climbing ability of the invasive vine *Lygodium microphyllum*. *Biol. Control*. 146: 104271.

David, A. S., I. M. Jones, and E. C. Lake. 2019. Wind speed predicts population dynamics of the eriophyid mite *Floracarus perrepae* on invasive Old World climbing fern (*Lygodium microphyllum*) in a shade house colony. *Exp. Appl. Acarol.* 78: 263–272.

DiTomaso, J. M., G. B. Kyser, J. R. Miller, S. Garcia, R. F. Smith, G. Nader, J. M. Connor, and S. B. Orloff. 2006. Integrating prescribed burning and clopyralid for the management of yellow starthistle (*Centaurea solstitialis*). *Weed Sci.* 54: 757–767.

Drus, G. M., T. L. Dudley, C. M. D'Antonio, T. J. Even, M. L. Brooks, and J. R. Matchett. 2014. Synergistic interactions between leaf beetle herbivory and fire enhance tamarisk (*Tamarix spp.*) mortality. *Biol. Control*. 77: 29–40.

Fellows, D. P., and W. E. Newton. 1999. Prescribed fire effects on biological control of leafy spurge. *J. Range Manag.* 52: 489–493.

FLEPPC. 2019. List of invasive plant species. Florida Exot. Pest Plant Coun. 1–2.

Fox, J., S. Weisberg. 2019. An R companion to applied regression, 3rd ed. Sage, Thousand Oaks, CA.

Goolsby, J. A., P. J. DE Barro, J. R. Makinson, R. W. Pemberton, D. M. Hartley, and D. R. Frohlich. 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Mol. Ecol.* 15: 287–297.

Goolsby, J. A., R. Zonneveld, and A. Bourne. 2004. Prerelease assessment of impact on biomass production of an invasive weed, *Lygodium microphyllum* (Lygodiaceae: Pteridophyta), by a potential biological control agent, *Floracarus perrepae* (Acariformes: Eriophyidae). *Environ. Entomol.* 33: 997–1002.

Hatcher, P. E., and B. Melander. 2003. Combining physical, cultural and biological methods: prospects for integrated non-chemical weed management strategies. *Weed Res.* 43: 303–322.

Herrera-Reddy, A. M., R. I. Carruthers, and N. J. Mills. 2012. Integrated management of scotch broom (*Cytisus scoparius*) using biological control. *Invas. Plant Sci. Manag.* 5: 69–82.

Hutchinson, J., A. Ferriter, K. Serbesoff-King, K. Langeland, and L. Rodgers (eds.). 2006. Old World climbing fern (*Lygodium microphyllum*) management plan for Florida, 2nd ed. South Florida Water Management District, West Palm Beach, FL.

Lake, E. C., and C. R. Minteer. 2018. A review of the integration of classical biological control with other techniques to manage invasive weeds in natural areas and rangelands. *BioControl.* 63: 71–86.

Lake, E. C., M. C. Smith, P. D. Pratt, A. J. Boughton, and R. W. Pemberton. 2014. Dispersal and establishment of new populations of the biological control agent *Floracarus perrepae* (Acariformes: Eriophyidae) on Old World climbing fern, *Lygodium microphyllum* (Polypodiales: Lygodiaceae). *Florida Entomol.* 97: 827–829.

Miller, T. W. 2016. Integrated strategies for management of perennial weeds. *Invas. Plant Sci. Manag.* 9: 148–158.

Mueller, R. J. 1982. Shoot morphology of the climbing fern *Lygodium* (Schizaeaceae): general organography, leaf initiation, and branching. *Bot. Gaz.* 143: 319–330.

Paynter, Q., and G. J. Flanagan. 2004. Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, *Mimosa pigra*. *J. Appl. Ecol.* 41: 615–629.

Paynter, Q., A. H. Gourlay, C. A. Rolando, and M. S. Watt. 2012. Dispersal of the Scotch broom gall mite *Aceria genistae*: implications for biocontrol. *New Zeal. Plant Prot.* 65: 81–84.

Pemberton, R. W. 1998. The potential of biological control to manage Old World climbing fern (*Lygodium microphyllum*), an invasive weed in Florida. *Am. Fern J.* 88: 176.

Pemberton, R. W., and A. P. Ferriter. 1998. Old World climbing fern (*Lygodium microphyllum*), a dangerous invasive weed in Florida. *Am. Fern J.* 88: 165.

Pyke, D. A., M. L. Brooks, and C. D'Antonio. 2010. Fire as a restoration tool: a decision framework for predicting the control or enhancement of plants using fire. *Restor. Ecol.* 18: 274–284.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sebesta, N., J. Richards, and J. Taylor. 2016. The effects of heat on spore viability of *Lygodium microphyllum* and implications for fire management. *Southeast. Nat.* 15: 40–50.

Slocum, M. G., W. J. Platt, and H. C. Cooley. 2003. Effects of differences in prescribed fire regimes on patchiness and intensity of fires in subtropical savannas of everglades national park, Florida. *Restor. Ecol.* 11: 91–102.

Stocker, R. K., R. E. Miller, D. D. Thayer, D. W. Black, and A. P. Ferriter. 2008. Using fire and herbicide to control *Lygodium microphyllum* and effects on a pine flatwoods plant community in South Florida. *Nat. Areas J.* 28: 144–154.

Tipping, P. W., M. R. Martin, and L. A. Gettys. 2017. Biological control increases the susceptibility of *Melaleuca quinquenervia* to fire. *Biocontrol Sci. Technol.* 27: 1014–1017.

Vieira, E. M., I. Andrade, and P. W. Price. 1996. Fire effects on a *Palicourea rigida* (Rubiaceae) gall midge: a test of the plant vigor hypothesis. *Biotropica.* 28: 210.

Wheeler, G. S., and K. M. Ordung. 2006. Lack of an induced response following fire and herbivory of two chemotypes of *Melaleuca quinquenervia* and its effect on two biological control agents. *Biol. Control.* 39: 154–161.

Zouhar, K., J. K. Smith, S. Sutherland, and M. L. Brooks. 2008. Wildland fire in ecosystems: fire and nonnative invasive plants in the northeast bioregion, gen. tech. rep. RMRS-GTR-42. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Ogden, UT.