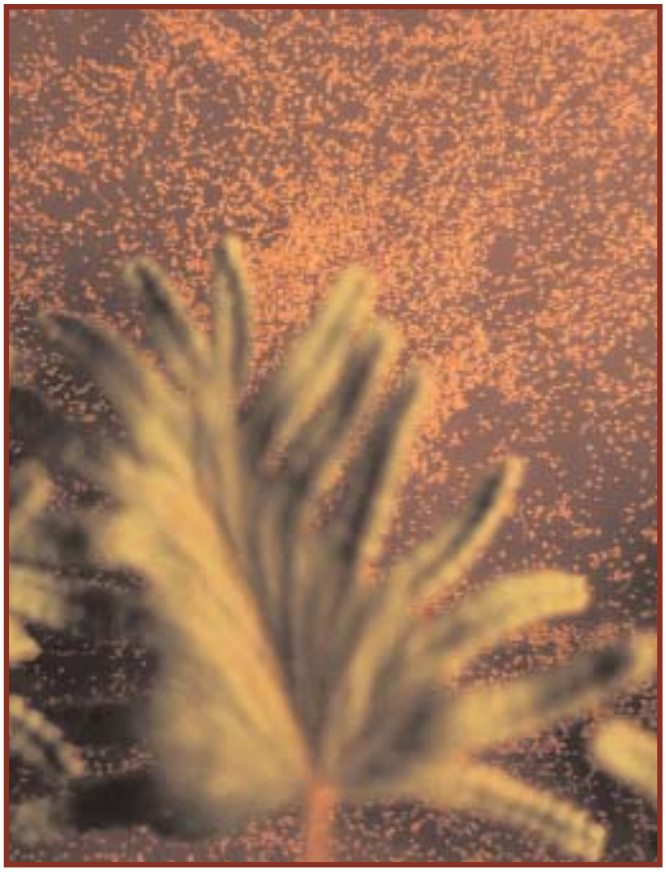

Fertile leaflets of Lygodium microphyllum *produce thousands of minute spores that can easily attach to the clothing and equipment of herbicide applicators.*

Potential Spread of *Lygodium microphyllum* Spores by Herbicide Applicators

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Old World climbing fern (*Lygodium microphyllum*) has spread across South Florida at a rapid rate relative to other invasive plants. This can be partly attributed to abundant wind borne spores that may be carried for miles, especially during major weather events such as tropical storms and hurricanes. A single fertile leaflet can produce thousands of spores, each one capable of starting a new fern population in an uninfested site (Lott et al. 2003).

Government agencies routinely hire contractors to treat Old World climbing fern infestations. Since contractors often work in multiple natural areas of the state, the potential exists for spores to spread to uninfested natural areas. The purpose of this project was to determine if herbicide applicators are spreading Old World climbing fern spores and, if so, to make recommendations on methods to limit the spread of spores by applicators.

METHODS

To quantify the amount of spores attached to applicators and equipment, we collected samples in the field using cotton squares in one re-treatment and three initial treatment sites infested with Old World climbing fern. Sampled sites included the Loxahatchee Slough in Palm Beach County (re-treatment site); initial treatment sites included Kissimmee River Restoration Site–Pool C in Highlands County, Allapattah Flats and Jonathan Dickinson State Park in Martin County. The re-treatment site had very low infestations of Old World climbing fern and had been treated within the past 12 months. Since infestations were low, samples were collected along the ground next to treated Old World climbing fern to determine if viable spores were still present one year after treatment. Initial treatment sites had heavy Old World climbing fern infestations with >75% vertical and horizontal coverage.

Cotton squares were used to collect samples from workers and equipment and were then placed in a covered petri dish with water. The samples were maintained under a temperature of 78ºF under cool florescent light for 16 hours and 8 hours of darkness. Samples were monitored at 20 and 40 days with a dissecting microscope for fern fragments and gametophytes (germinated spores). Old World climbing fern fragments found on the squares were observed to contain spores and represent micro-debris that can easily detach from clothing and equipment. Gametophytes are viable spores and, once fertilized, become sporophytes. Gametophytes are easily detected under a dissecting microscope.

Means, standard errors, range, and 95% confidence intervals were calculated for all samples. Initial and re-treatment sites were tested for differences between the number of gametophytes for all samples using a Mann-Whitney Test. Differences among samples were computed for both re-treatment and initial sites using a Kruskal-Wallis test and differences among sample types were tested with Multiple Comparison Test (Noether 1990).

RESULTS

Samples taken from workers and equipment at re-treatment and initial treatment sites contained micro-fragments of Old World climbing fern and gametophytes (Table 1). Spores were commonly observed attached to fern micro-fragments in many samples, but no quantitative data was collected. The mean number of gametophytes for all samples at the re-treatment site was 3.5 (SE = 0.8; Range 0 – 32), and 173.2 (SE = 42.3; Range 0 – 1472) for initial treatment sites. Significant differences were detected for the number of gametophytes between re-treatment and initial treatment sites ($Z = -8.338$, $P < 0.01$).

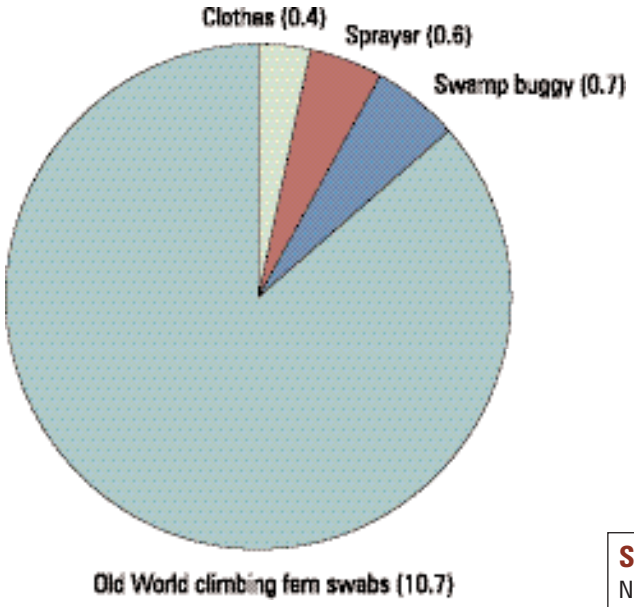
The average number of gametophytes per sample at re-treatment (low infesta-

Herbicide applicators taking samples for spores of *Lygodium microphyllum*.

PRACTICE GOOD SPORE HYGIENE

- Prior to leaving site, thoroughly spray down all equipment with a high-pressure sprayer using either water or compressed air.
  - Focus on all openings, cracks, crevices, and treads, including the underside of vehicles.
  - Cleaning of equipment should occur along the edge of the infestation to avoid spreading spores to areas outside the infested site.
- Clothing and accessories (shoes, gloves, hats, etc.) should be brushed off on-site to remove Old World climbing fern fragments and spores.
- Wash all clothing daily.
- Disposable suits should be removed prior to leaving the infested site and placed in a plastic bag.
- Limit track vehicles and swamp buggies in areas heavily infested with Old World climbing fern.
  - Treaded tires and tracks from vehicles easily trap soil that may contain spores.
  - Vehicles are difficult to clean due to the many crevices, openings, and covered parts.

Fig. 1 – **Low Infestation**



Spore contamination
Numbers in parentheses represent the average number of gametophytes detected per sample.

Fig. 2 – **High Infestation**

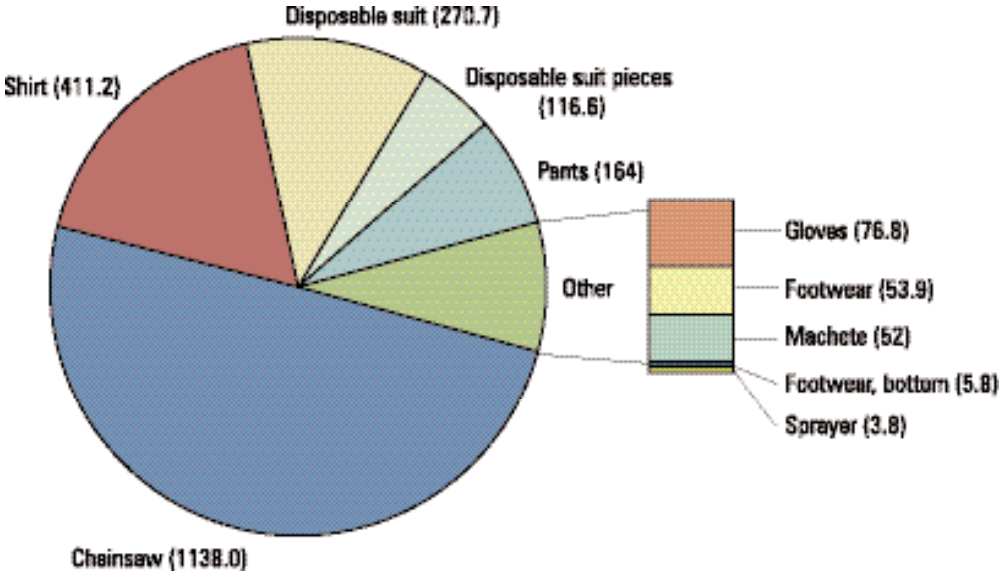


Table 1. Percentage of Old World climbing fern micro-fragments and gametophytes from samples taken from re-treatment (low infestation) and initial treatment (heavy infestation) sites.

	Old World climbing fern	
	Fragments	Gametophytes
Re-treatment site (n = 1)	22.2 %	56.8 %
Initial treatment site (n = 3)	61.6 %	96.1 %

tion) sites was < 1.0 for clothing, sprayers, and a swamp buggy, and no significant difference was detected among samples (Fig. 1). Samples taken from Old World climbing fern rachis mats treated 12 months prior at the re-treatment site averaged 10.7 gametophytes, and was significantly different than clothing, sprayers, and swamp buggy samples ($H = 42.2$, d.f. = 3, $P < 0.01$).

Within initial treatment (heavy infestation) sites, the average number of gametophytes for each sample varied between 3.8 (S.E. = 1.4) for sprayers and 1138.0 (S.E. 199.8) for chainsaws (Fig. 2). Clothing contained a higher, but not significant, number of gametophytes than equipment, with the exception of chainsaws. The highest number of gametophytes on clothing were detected on pants (mean = 164.0, S.E. = 87.0), disposable suits (mean = 270.7, S.E. = 103.7), and shirts (mean = 411.2, S.E. = 215.4). Significant differences were detected among samples from sprayers and the bottom of footwear compared to other samples at initial treatment sites ($K = 26.9$, d.f. = 9, $P = 0.001$).

DISCUSSION

Spores of Old World climbing fern were found to easily contaminate clothing and equipment. Even in re-treatment sites with light infestations and no fertile leaflets, gametophytes were detected in 56.8% of the samples. Samples taken with cotton swabs through Old World climbing fern treated one year prior averaged 10.7 gametophytes, indicating that viable spores are present at least one year after treatment.

In heavily infested sites, clothing contained a higher, but not significant, number of gametophytes than equipment, with the exception of sprayers and bottom of footwear. The large variation among samples may be explained by the treatment method wherein some workers cut the fern and other workers spray the cut portion of the fern. Workers cutting the fern are more likely to come in contact with fertile leaflets and falling spores than workers applying herbicide. Samples were not distinguished between workers cutting or spraying, but samples from two different applicator's shirts from the Kissimmee River site contained 95 and 1219 gametophytes, which may be attributable to one worker cutting and one spraying the plant. Due to this large variation in the number of gametophytes among samples taken from workers, all workers should take precautions to limit spreading spores to other areas.

Researchers and others working around Old World climbing fern also should clean their clothing and equipment prior to leaving the site. Samples taken from a researcher working in a heavily infested site revealed an average of 31 and 142 gametophytes for footwear and pants, respectively. While aggressive treatment of Old World climbing fern should not be hindered by decontamination of clothing and equipment, we do have some suggestions.

Please see "Practice Good Spore Hygiene" on page 14.

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