

Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

*Biological
Control*

IMPLEMENTATION AND STATUS OF BIOLOGICAL CONTROL OF THE HEMLOCK WOOLLY ADELGID



United States
Department
of Agriculture



Forest
Service



Forest Health
Technology
Enterprise Team

FHTET-2011-04
December 2011

The Forest Health Technology Enterprise Team (FHTET) was created in 1995 by the Deputy Chief for State and Private Forestry, Forest Service, U.S. Department of Agriculture, to develop and deliver technologies to protect and improve the health of American forests. This book was published by FHTET as part of the technology transfer series.

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On the cover:

Center photo: Hemlock woolly adelgid white woolly masses on a hemlock branch (USDA Forest Service, Karen Felton)

Top right: *Sasajiscymnus tsugae* predatory beetle (USDA Forest Service, Lynn Jones);

Middle right: Collecting and checking hemlock branch samples for *Laricobius nigrinus* predatory beetle larvae (USDA Forest Service, Brad Onken);

Bottom center: Collecting *Laricobius nigrinus* predatory beetles in Idaho (USDA Forest Service, Brad Onken);

Middle left: Releasing *Laricobius nigrinus* predatory beetles (USDA Forest Service, Brad Onken);

Top left: *Laricobius nigrinus* predatory beetle (USDA Forest Service, Lynn Jones)

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IMPLEMENTATION AND STATUS OF BIOLOGICAL CONTROL OF THE HEMLOCK WOOLLY ADELGID

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PREFACE

Hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), remains the single greatest threat to the health and sustainability of hemlock as a forest resource in the eastern United States. It is an exotic pest native to Asia and western North America. First discovered in the eastern United States in 1951 near Richmond, VA, the pathway and source of the introduction are believed to have originated from southern Japan on nursery stock. In the late 1980s, HWA began to spread rapidly from its point of introduction. It has since spread to 17 eastern states threatening two species of hemlock: the eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *Tsuga caroliniana* Engelm. On these hemlocks, HWA has two generations per year and is parthogenic; that is, all individuals are female, capable of reproducing.

The Hemlock Woolly Adelgid Initiative began in 2003 with the development of a 5-year program (2003-2007) in cooperation with the U.S. Department of Agriculture Forest Service and the Animal Plant Health Inspection Service (APHIS), the National Association of State Foresters, and the National Plant Board for the development and

implementation of management options to reduce the spread and impact of HWA. A second 5-year program (2008-2012) was funded to continue and to accelerate the development of research and technology, management, and information transfer program components.

Although the earliest investigations into HWA biological control date back to the early 1990s, the biological control effort has been greatly expanded with the onset of the Hemlock Woolly Adelgid Initiative and has involved 28 federal and state agencies, 24 universities, 7 institutions in China and Japan, and numerous private industries. Biological control offers a potential long-term solution to suppression of HWA and needs to become the focal component of an integrated management program.

The purpose of this book is to provide a reference guide for field workers and land managers on the historical and current status of the biological control of hemlock woolly adelgid. This book is a substantial revision of FHTET-2004-04, *Biological Control of Hemlock Woolly Adelgid*.

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CHAPTER 11: REARING LABS AND DISTRIBUTION OF PREDATORS FOR RELEASE

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REARING LABS

There are currently eight primary labs rearing five different predators of hemlock woolly adelgid. The labs include Clemson University, New Jersey Department of Agriculture (Philip Alampi Beneficial Insect Laboratory), North Carolina Department of Agriculture and Consumer Services, North Georgia College & State University, University of Georgia, University of Tennessee (Lindsay Young Beneficial Insect Laboratory), Virginia Tech, and Young Harris College. Species currently being reared include *Laricobius nigrinus* Fender, *Laricobius osakensis* Montgomery and Shiyake, *Scymnus coniferarum* Crotch, *Scymnus sinuanodulus* Yu et Yao and *Sasajiscymnus tsugae* Sasaji and McClure. Table 1 details the species currently being reared by each lab as of fall 2011. This has been a dynamic list and will likely continue to change.

DISTRIBUTION OF PREDATORS FOR RELEASE

Predators of hemlock woolly adelgid have been released across 17 states in the Eastern United States. Operational releases began as early as 1998 and have continued steadily since then. Releases take place primarily on public lands, including state and national forests, parks and Hemlock Conservation Areas. Figure 1 depicts release locations of three primary predators (*S. tsugae*, *S. sinuanodulus*, and *L. nigrinus*) which have been entered into the newly created HWA Predator Release and Monitoring Database. Entry of this data is currently ongoing and many releases have yet to be represented. Additional species will also be added.

Table 1. Rearing labs

Rearing Lab	L.n.*	L.o.	S.c.	S.s.	S.t.
Clemson University	x				x
New Jersey Department of Agriculture	x				
North Carolina Dept. of Agriculture and Consumer Services					x
Northern Georgia College & State University					x
University of Georgia	x			x	
University of Tennessee	x	x			x
Virginia Tech	x	x	x		
Young Harris College					x

*L.n. = *Laricobius nigrinus* Fender; L.o. = *Laricobius osakensis* Montgomery and Shiyake; S.c. = *Scymnus coniferarum* Crotch; S.s. = *Scymnus sinuanodulus* Yu et Yao; S.t. = *Sasajiscymnus tsugae* Sasaji and McClure

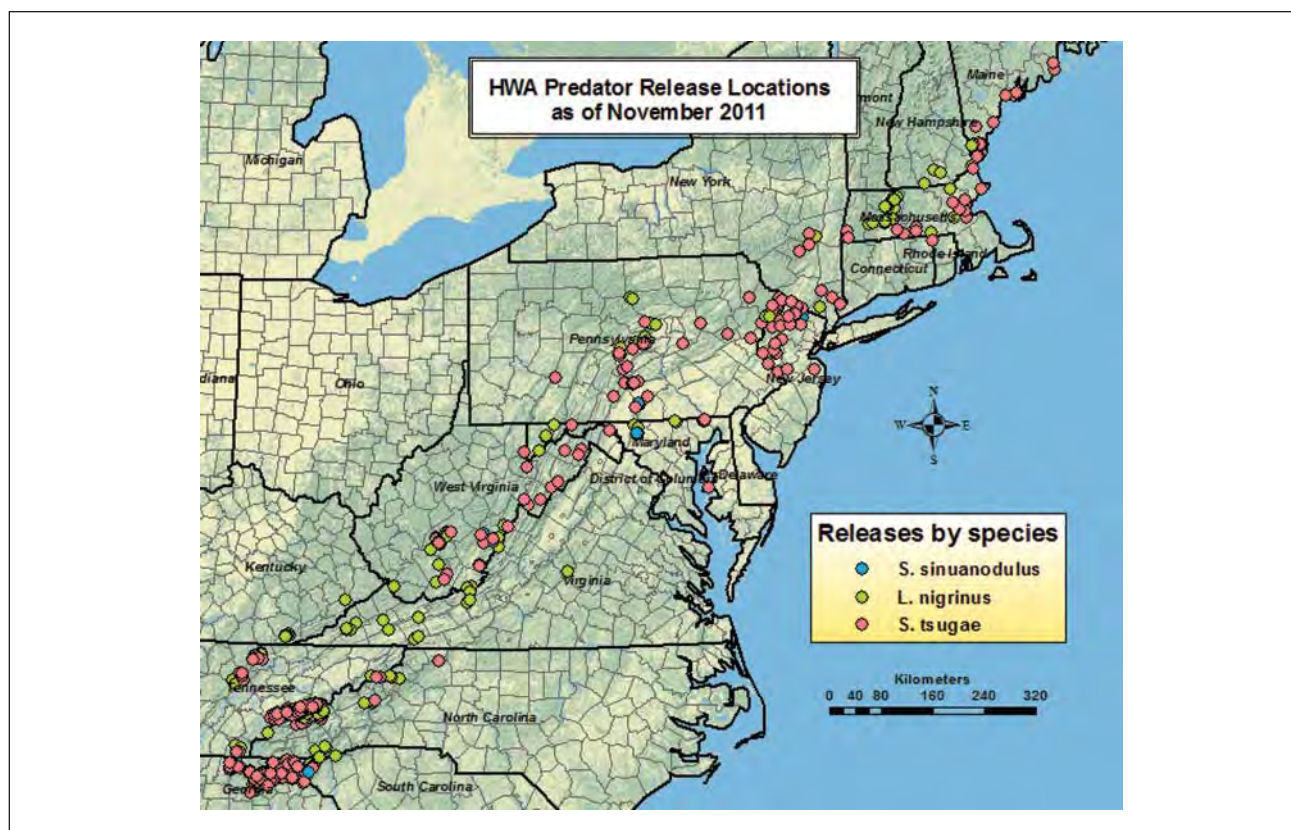


Figure 1. Map of the Eastern United States showing *S. tsugae*, *S. sinuanodulus*, and *L. nigrinus* releases which have been entered into the HWA Predator Release and Monitoring Database.

***Laricobius nigrinus* Release Locations**

Clemson: Georgia, North Carolina, and South Carolina

New Jersey Department of Agriculture: Pennsylvania and New Jersey

University of Georgia: U.S. Forest Service Hemlock Conservation Areas throughout Georgia

University of Tennessee: Tennessee – Great Smoky Mountains National Park, Cherokee National Forest; Tennessee State Parks and Natural Areas, and Tennessee Wildlife Management Areas

Virginia Tech: Connecticut, Georgia, Maine, Maryland, Massachusetts, New Hampshire, New York, North Carolina, Pennsylvania, Rhode Island, Vermont, Virginia, West Virginia

***Sasajiscymnus tsugae* Release Locations**

Clemson University:

South Carolina - Sumter National Forest, Jocassee Gorges, Mountain Bridge Wilderness Area, Oconee State Park;

North Carolina – Nantahala National Forest

Georgia – Chattahoochee National Forest

New Jersey Department of Agriculture:

Connecticut, Maryland, Massachusetts, Maine, New Hampshire, New Jersey, North Carolina, New York, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia

North Carolina Department of Agriculture and

Consumer Services: North Carolina, Maine

North Georgia College & State University:

Georgia – U.S. Forest Service Hemlock Conservation Areas throughout North Georgia on National Forest and State lands

University of Georgia: U.S. Forest Service Hemlock Conservation Areas throughout Georgia

University of Tennessee:

Tennessee – Great Smoky Mountains National Park, Cherokee National Forest; Tennessee State Parks and Natural Areas, and Tennessee Wildlife Management Areas

Georgia – U.S. Forest Service study

Young Harris College:

Georgia – Chattahoochee National Forest and State properties in North Georgia

***Scymnus sinuanodulus* Release Locations**

Clemson University: Chattooga River – Bull Sluice Site (Georgia/South Carolina border)

New Jersey Department of Agriculture: Maryland, New York, North Carolina, Pennsylvania, Tennessee, Vermont, West Virginia

Northern Georgia College & State University:

Georgia – Hemlock Conservation Areas throughout North Georgia on National Forest and State lands

University of Georgia: U.S. Forest Service Hemlock Conservation Areas throughout Georgia

University of Tennessee: Tennessee – Great Smoky Mountains National Park

***Scymnus coniferarum* Release Locations**

No releases yet.

A summary of release totals is shown in Table 2.

REARING PROCEDURES***Laricobius nigrinus***

Dr. Ashley Lamb developed *Laricobius nigrinus* rearing procedures at Virginia Tech from 2000-2005 while working toward her Ph.D. and later as a postdoctoral associate. They were adapted

for mass rearing, and have since been adjusted to maximize production. Each lab has manipulated their rearing protocols to fit their individual facility limitations such as space availability and equipment. In general, mass rearing begins in late January or early February when both HWA and *L. nigrinus* begin to lay eggs. New adults are collected each year in their native habitat, the Pacific Northwest area of the United States. These adults are brought back to rearing labs and serve as founders for that year's colony. Collecting fresh beetles each year helps add to the genetic diversity of released populations. Additionally, wild-caught beetles are often more fecund which results in higher laboratory production numbers. Once adult *L. nigrinus* beetles are brought back to the lab, they are held in clear, plastic feeding containers on hemlock bouquets (Fig. 2). These bouquets usually consist of roughly 20-25 branches of highly infested hemlock roughly 20-25 cm in length. These are nested in Parafilm wrapped floral foam for hydration.

Adults are randomly placed in containers, since sex cannot be determined through visual observation. They are held at densities of roughly 20 beetles per container to ensure minimal competition between individuals for feeding and egg laying sites. Adults will remain on these bouquets for a

Table 2. Release totals

Rearing Lab	<i>L. nigrinus</i>	<i>S. coniferarum</i>	<i>S. sinuanodulus</i>	<i>S. tsugae</i>
Clemson University	14,605 (adults) + 44,678 (eggs)		52 (adults)	940,721 (adults) + 82,256 (eggs)
New Jersey Department of Agriculture	75 (adults)		23,348 (adults)	536,290 (adults) + 22,744 (eggs) + 360 (larvae)
North Carolina Dept. of Agriculture and Consumer Services				484,800 (adults)
Northern Georgia College & State University			2,080 (adults)	204,206 (adults)
University of Georgia	141,580 (eggs)		32,089 (eggs)	Minimal egg releases made in 2006
University of Tennessee	17,656 (adults)		98 (adults)	736,733 (adults) + 89,084 (eggs)
Virginia Tech	72,489 (adults)			
Young Harris College				120,034 (adults) + 100,092 (eggs)

period of one week at specific temperatures and daylength depending on the time of year (Fig. 3).

Adults are removed after one week and the foliage is placed into floral foam blocks with fresh twigs. These blocks are then nested into galvanized steel funnels or other larval rearing systems where eggs are allowed to hatch and subsequent larvae develop (Fig. 4).

If labs are releasing eggs, they are placed in the field at this time. Typically, temperatures during this phase of development are between 12-15 °C with a 12:12 L:D daylength. It takes approximately 4-6 weeks for larvae to hatch and develop through 4 instars. Since their natural tendency is to drop to the soil when development is complete, larval rearing systems are designed to have a catch area such a drawer or jar under the foliage, where the larvae can congregate. These are checked daily until roughly July when production typically stops.

Collected larvae are immediately placed into summer aestivation containers. These containers are relatively small (approximately 15 cm × 15 cm) plastic, and ventilated on at least two sides for adequate air circulation (Fig. 5).

Approximately 6-7.5 cm of soil is placed into these containers with moisture levels at or close to 35% since the pre-pupae and pupae prefer a wet soil. A 2:1 ratio of peat moss and sand is used to create the most optimal soil mix. Mature larvae are simply placed on the soil surface and they eventually burrow down to create a pupation cell. Once in the soil, containers are held at roughly 12-15 °C for approximately 6 weeks while pupae develop into adults.

After pupae eclose, containers are then moved into 19 °C (16:8 L:D) temperatures for the summer months. These higher temperatures again mimic conditions in their native habitat and encourage the adults to stay in the ground. Soil moisture levels are kept very low at this point. Containers are watered very lightly on a weekly basis.



Figure 2: *L. nigrinus* feeding containers.

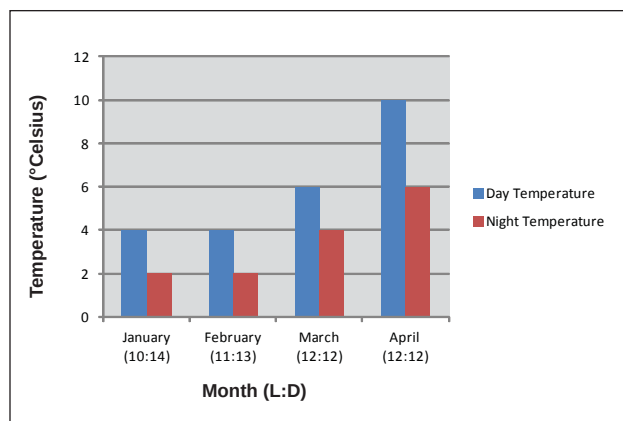


Figure 3. *L. nigrinus* adult feeding temperatures and daylength during oviposition.



Figure 4: *L. nigrinus* larval rearing system utilizing steel funnels.

When HWA has broken its summer diapause in the field in early October, *L. nigrinus* aestivation containers are then moved to lower temperatures to encourage emergence (12-15°C; 12:12 L:D). Adults will typically begin emerging in large numbers about 2 weeks after the temperature is dropped and will continue to emerge from these containers until mid to late December. If labs are doing adult releases, they are typically placed in the field during this time.

Laricobius osakensis

Protocols for the mass rearing of *L. osakensis* are currently being developed at Virginia Tech. Dr. Ashley Lamb has completed initial groundwork, which is being followed by Ph.D. student Ligia Cota Viera and the Virginia Tech mass rearing staff. Many procedures will be identical to that of *L. nigrinus*; however, oviposition temperatures will likely be slightly lower.

Sasajiscymnus tsugae

Carole Cheah and the staff at the Connecticut Agricultural Experiment Station (CAES) developed initial small-scale rearing procedures for *S. tsugae*. These methods were then built upon and adjusted for mass rearing by Dan Palmer and Jennifer DeSio at the New Jersey Department of Agriculture (PABIL) and now stand as the foundation for procedures utilized at other rearing labs today. Each lab has tweaked protocols to suit their facilities'



Figure 5. *L. nigrinus* soil aestivation containers.

capabilities and limitations. Ovipositioning adults are typically held on hemlock bouquets placed in soaked floral foam in 1 gallon glass jars with modified, ventilated lids. Eight to ten twigs, 15-20 cm in length, comprise one bouquet (Fig. 6).

Gauze is placed between foliage to help estimate egg totals, as roughly 50% of the total number of eggs will be laid there. Beetles are typically held at densities of 15 per container with a 10:5 female: male ratio. Honey is added as a supplemental diet. Glass jars are held on their sides on racks at 24-25 °C, 55-65% RH, 16:8 L:D (Fig. 7).

Adults are removed after one week and the foliage and gauze are placed into a rearing cage. Adults are placed on fresh bouquets and the process is repeated. If egg releases are being made and if outdoor temperatures are consistently above 15 °C, they are done at this time.



Figure 6. *S. tsugae* oviposition jars.



Figure 7. *S. tsugae* oviposition jars on racks.

Foliage and gauze containing eggs are placed in rearing tents (BugDorm – 152 cm × 152 cm × 152 cm) until an estimated 1500 eggs (750 from gauze) is reached (Fig. 8).

Twigs are placed into foam blocks with the gauze sandwiched between. Fresh, infested hemlock branches are added 2 times per week and after 20 days a honey strip is placed in each cage 1 time per week.

Any adults found during this time are collected and placed into storage cages. Rearing cages are broken down after roughly 30-50 days. All foliage is examined and any beetles found are collected. Up to 400 adults are placed into storage cages at 18-20 °C, 55-65% RH, 16:8 L:D at least 3 weeks prior to release to ensure they are sexually mature. Adult releases begin when outdoor temperatures stay above freezing.



Figure 8. BugDorm rearing tents.

Adults are stored in summer with protocols similar to those used for the rearing cages. Roughly 400 adults are held in each cage at temperatures of 14-20 °C, 55-65% RH, 12:12 L:D, and foliage blocks are replaced every 45-60 days.

Scymnus sinuanodulus

Since it is so similar to *S. tsugae*, protocol development for the mass rearing of *S. sinuanodulus* was again based on the foundations built by Carole Cheah at CAES and Dan Palmer and Jennifer DeSio at PABIL. Also, as with *S. tsugae*, individual labs have tweaked various procedures to meet their particular needs. More specifically, a multi-lab collaborative effort to fine-tune protocols for operational egg releases was headed up by the staff at the University of Georgia rearing facility. Rearing begins in February after HWA is confirmed to be developing eggs in the field. Adults are held at densities of 9 (6:3 female:male) on 5 twigs of infested hemlock. Temperatures run from roughly 12-14 °C, RH 65%, 12:12 L:D. Gauze is also used as egg indicator in oviposition jars but *S. sinuanodulus* will only lay roughly ¼ of its eggs there. Twigs are removed and refreshed weekly through June. Egg releases are done with the resulting twigs. If rearing to the adult stage, resulting twigs will be placed in BugDorms for larval development. The use of ample quantities of fresh, heavily infested hemlock is critical since larvae can become cannibalistic. Fresh foliage is added weekly. Larval rearing temperatures are set to 20-22 °C, 65% RH and 16:8 L:D. Adults will begin to appear in 7-8 weeks. They are collected and stored at 20-22 °C for 4 weeks at 16:8 L:D, 65% RH. These conditions allow maturation and mating to occur. They are then moved to 12-14 °C at higher densities (50-60 adults) until October when beetles are released.

Scymnus coniferarum

S. coniferarum is currently being studied in quarantine at Virginia Tech to confirm suitability as a biological control agent of HWA. Preliminary rearing techniques are being developed; however, rearing will likely be similar to other *Scymnus* species. Adults will typically lay more eggs at 26 °C but will die off sooner. Adults are therefore

held at 18-20 °C as a compromise. They're held at densities of roughly 12 with a 2:1 female:male sex ratio, although it is difficult to distinguish between sexes. Gauze is also used in oviposition bouquets to estimate the number of eggs laid. Larvae are reared at 18 °C and adults emerge in roughly 50 days at this temperature.

GENERAL ISSUES AND PRODUCTION LIMITATIONS

Mortality before Release (*S. sinuanodulus* and *S. tsugae*)

Adults are typically held for several weeks before release to allow for mating and feeding; however, mortality is often high during this time.

Early Emergence (*L. nigrinus*)

A significant challenge when rearing *L. nigrinus* is that of early emergence of adults before HWA has broken diapause in the field. *L. nigrinus* adults will not feed on aestivating HWA nymphs so a great deal of mortality can occur if beetles are emerging from the soil early in the lab. A small level of early emergence typically occurs at all labs each year; however, some years have been particularly high. This is thought to happen when *L. nigrinus* larvae do not receive ample nutrients during their maturation process. Other unknown factors likely play into this as well. Mortality levels can be devastating. Holding early-emerged adults at low temperatures and offering supplemental artificial diets helps to stave off high mortality levels before HWA breaks. Holding temperatures of at least 4 °C greatly increased survival.

Staffing

High turnover rates have been shown to negatively affect success rates at the various labs. The rearing of each species is very labor intensive and challenging. *L. nigrinus*, for example, only has one generation per year. It takes several seasons to fully understand what is necessary to be successful. With high turnover, you lose that gained knowledge and must start over with training. Some labs also have trouble finding funding to hire ample staff (Fig. 9).



Figure 9. Rearing lab staff.

Equipment/Rearing Space

Because biocontrol agents of HWA are winter active, this presents a unique challenge for rearing in terms of temperatures. Some predators require temperatures as low as 4 °C (39 °F) for extended periods of time. Temperatures of roughly 12 °C (54 °F) must be available in large spaces for rearing of *L. nigrinus* larvae. Most rearing facilities have faced the challenge of retrofitting old buildings and equipment to create these large spaces. Even with the best equipment, there are failures, which can cause temperature spikes or drops. These fluctuations can be devastating to colony health. Several labs have seen high mortality due to this reason.

Food Quality

As the HWA infestation front moves westward and trees continue their rapid decline, it has become increasingly difficult to find heavy adelgid infestations on relatively healthy trees with which to feed colonies. Significant travel time is involved for some labs to find the best infestations, often requiring overnight trips. Other labs must have food shipped in, which presents problems in terms of quality of prey health. Since such a high volume of food is necessary for rearing, especially in the spring, this becomes the primary challenge for most labs (Fig. 10).

Pathogens

As touched upon in other chapters of this publication, pathogens such as microsporidia are



Figure 10. Infested hemlock used for predator rearing.

a significant issue for rearing predators in the lab. Colonies are routinely tested for the presence of microsporidia since this organism is found occurring naturally in wild populations. Since most *L. nigrinus* rearing begins with yearly introductions of insects collected in the wild, it is important to do these tests. Infections in the lab can be devastating to fecundity levels and very difficult to eradicate.

ACKNOWLEDGEMENTS

Paul Arnold, LayLa Burgess, Carole Cheah, Hugh Conway, Mark Dalusky, Jen DeSio, Cera Jones, Kathy Kidd, Ashley Lamb, Pat Parkman, Andy Roberts, Ligia Cota Viera.

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