Diagnosing the presence of *Sirex noctilio* from examination of dead and dying pine trees

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Abstract. A growing challenge in applied entomology is to develop sampling techniques for new invasive species. The European woodwasp, *Sirex noctilio*, was discovered in New York State in 2004. This species can kill pine trees and is a potentially significant pest of North American pines. The distribution of *S. noctilio* in North America is apparently expanding, but is only approximately known and there is great uncertainty as to speed and directions of expansion. Landowners, entomologists, and foresters in potentially affected regions may wish to survey for the presence of *S. noctilio*. Unfortunately, the adults are seasonally ephemeral and individuals of any life stage are difficult to detect by any means even when present. Here we identify diagnostic symptoms of stand occupancy by *S. noctilio*, and suggest a protocol for rapid inexpensive assessment that can be performed at any time of year by examination of dead and dying pine trees. Done carefully, such surveys can sometimes reliably rule out the presence of *S. noctilio* as a source of tree mortality within any particular stand or can yield evidence of their presence that is sufficiently compelling to warrant additional sampling. The process involves examining the shape, size, number, and spatial patterning of emergence holes. The characteristic features persist for many years even after the log is on the ground. This increases the likelihood of detection where *S. noctilio* is present, strengthens the inference of non-occupancy from searches that fail to detect *S. noctilio*, and can also suggest the duration of occupancy in a region.

Keywords: *Amylostereum*, detection, invasive species, *Monochamus*, *Pinus resinosa*, *Pinus sylvestris*, woodborer

Citation for this document:
Introduction

Accelerating introductions of non-indigenous insect pests are among the greatest threats to natural and managed ecosystems in North America and throughout the world (Seppälä et al. 2009, Yemshanov et al. 2009). Efficient sampling for potential invasives is among the prominent challenges for contemporary entomology. Following the initial detection of new and potentially damaging insect species it becomes important to determine the extent of occupancy, the rate and direction of range expansion, and the abundance within occupied regions (Yemshanov et al. 2010). This knowledge is essential for evaluating, and potentially implementing, management tactics such as eradication, quarantines, slow-the-spread programs, or acceptance and adaptation (Koch et al. 2009).

Standardized insect trapping programs are by the far the most common technique for detecting and monitoring introduced insects. Examples include semiochemical-based trapping and aerial suction traps (Harrington and Woowd 2007, Tobin et al. 2009). These can be very efficient when there is an attractive lure and/or if the insects are quite abundant when present, but this is not always the case. For example, Asian long-horned beetle and emerald ash borer have provided vexing problems for detection systems in North America because long distance pheromones are not known (and may not exist) and attractant host volatile blends with which to bait traps are elusive (Colunga-Garcia et al. 2010, Gandhi and Herms 2010, Marshall et al. 2010, Nehme et al. 2010). An alternative tactic is to search for diagnostic symptoms of the insects. This alternative will be most practical when large areas can be searched (e.g., via remote sensing; Mutanga and Ismail 2010) or if the diagnostic symptoms are persistent throughout the year and across years and unique to a given species.

The European woodwasp, *Sirex noctilio* Fabricius, is a notable new example of a challenging insect detection problem in North America. This species is native to Europe where it has seldom been reported to produce notable damage (Dajoz 1980, Wermelinger et al. 2008), but has become an important pest in pine plantations of the southern hemisphere following accidental introductions (Carnegie et al. 2006, Hurley et al. 2007, Iede and Zanetti 2007). In North America, it was first detected in Oswego county, New York in 2004, and is thought to have entered within wooden palettes on cargo ships from Europe that entered Lake Ontario via the St. Lawrence Seaway (Nielsen et al. 2009, USDA APHIS 2010). This triggered a continuing geographically broad sampling program to determine the extent of occupancy and rate of range expansion (USDA APHIS 2010, Dodds and de Groot 2011). In 2005-2006, it was detected in 24 other counties in New York State and two counties in northern Pennsylvania. In 2007 it was detected in four more counties in New York, four more counties in Pennsylvania, and one county each in eastern Michigan and north central Vermont. In 2008-2009, it was detected in two more counties in Pennsylvania and three more in Michigan. During the same period it has been detected in an expanding area of southern Ontario (Dodds et al. 2010). However, the full extent of the distribution in North America is not known, nor is it known how many of the new detections represent continuing range extensions versus detection of previously existing populations. Furthermore, the trapping provides little information regarding local abundance. This is because detection of *S. noctilio* has proven to be another difficult sampling problem. Distance pheromones have not been discovered (Boroczky et al. 2009). Lures based on host volatiles catch some *S. noctilio*, but with very low yield. Even traps placed on experimentally stressed trees within areas known to have well established populations produced few captures of *S. noctilio* (Zystra et al. 2010). An alternative sampling tactic, which is more likely to detect *S. noctilio* when it is present, is to employ trap trees, but this involves the use of regulated herbicides to kill trees, chainsaws to later cut logs, and
large containers for subsequently rearing insects from the logs (Zylstra et al. 2010). While trap trees provide several opportunities to detect *S. noctilio* (distinctive resin beading on tree boles, adult captures in traps, log dissection to obtain larval samples, or rearing to obtain adult specimens), it is probably too expensive and complicated for widespread use.

Here we describe techniques for an alternative survey technique that could be employed by any interested person with relatively little time, training, or expense. The process is to examine multiple dying and recently dead pine trees within a stand or region, and to search for resin beads diagnostic of *S. noctilio* while also examining the size, shape, number, and spatial patterning of emergence holes within and among trees.

**Materials and Methods**

During spring and summer 2008, working near Syracuse, New York, we used a log splitter to examine the insect feeding galleries within the xylem of >200 logs (55 cm in length, 20-30 cm diameter) from pine trees that had been chemically girdled in June 2007 and subsequently colonized by *Sirex noctilio* as well as other wood boring insects. These logs were part of the study reported by Zylstra et al. (2010). From a sample of 26 logs from 19 different pine trees (10 and 9 red pine and scots pine, respectively), we systematically measured the diameter of each emergence hole (*n* = 162), marked the holes, and then carefully split each log into as many pieces as necessary to determine which holes were associated with feeding galleries of *S. noctilio* vs. woodborers (Cerambycidae or Buprestidae). From the larger set of logs in this study, we identified and sexed 729 individual *Monochamus* spp. (Cerambycidae) to determine the identity of the species in this area that produced emergence holes most easily confused with those of *S. noctilio*.

We also examined large numbers of dead and dying trees for symptoms of occupancy by Siricidae. within stands of hard pine (red or scots pine) within and outside the areas known to be infested by *S. noctilio*. During summer 2008, we examined on foot 31 pine stands in Oswego and Onondaga counties, New York. During summer 2009, we examined 23 stands in the Adirondack region of New York, 8 stands within Finger Lakes National Forest (FLNF), and 8 stands in Tioga county, Pennsylvania (Appendix). We examined both standing trees and fallen logs for emergence holes that could have been produced by Siricidae. We employed hatchets as needed to expose enough of the feeding gallery to distinguish between emergence holes that were associated with Siricidae vs. *Monochamus* spp. We used close-focus binoculars to examine regions of tree boles that were out of reach.

Within one infested stand of Scots pine in Schuyler County, NY (site 132), we systematically examined one face of the bole of 45 standing, recently dead Scots pine with close-focus binoculars and counted the occurrence of Siricidae emergence holes. Counts were repeatable between two observers at $r^2 = 0.98$.

**Results and discussion**

**Size and shape of emergence holes.**

The emergence holes of *S. noctilio* appear to be perfectly round, presumably because the animals are spiraling as they bore through the xylem (Fig. 1, left). The internal feeding galleries contain very fine, lightly colored, frass that appears as sawdust tightly packed behind the larvae (Fig. 1, right). The holes most easily confused with that of Siricidae are those produced by woodboring beetles (especially Cerambycidae). With some care, and consideration of several criteria, the holes produced by Siricidae can be reliably distinguished from those produced by woodborers. Even at some distance up the bole of a tree, woodborer exit holes are usually visibly non-circular (elliptical or D-
shaped). However, even after we have looked at thousands of *Sirex* holes, we still find some emergence holes from woodboring beetles that we would classify as Siricidae-like based just on the circularity. The size and patterning of holes provides additional criteria.

The diameter of *Sirex noctilio* emergence holes (verified by splitting the logs to expose the galleries) ranged from 0.5 to 6.0 mm (N = ?; mean ± SD = 3.4 ± 0.9 mm; Fig. 2 lower). The same logs contained 38 emergence holes of similarly sized woodboring beetles, about 20% of which were not clearly distinguishable to us from Siricidae holes based simply on the circularity of the hole and angle of exit. These non-Siricidae holes were chiefly produced by *Monochamus* spp., which accounted for the vast majority of non-Siricidae woodboring insects produced from the trap trees: 78% of 994 adults that we examined were *M. carolinensis* (Olivier) and the remainder were *M. scutellatus* (Say), with ≈1:1 sex ratios for both species. Emergence holes of *Monochamus* spp. were larger on average but overlapped with those of *S. noctilio* (mean diameter ± SD = 5.5 ± 0.87 mm, range = 3.0 to 7.1 mm; Fig. 2 upper). Helpfully, 40% of the *S. noctilio* holes were smaller than the minimum diameter of *Monochamus* holes (3.0 mm). These smaller *S. noctilio* holes were still larger than those of ambrosia beetles (exit holes < 1.5 mm diameter), which also exit from the xylem. Thus, at least in this sample, round exit holes of 1.5-3.0 mm were diagnostic of Siricidae (although not necessarily *S. noctilio*).

Independent measurements of the head capsule width of 110 adults of *Sirex noctilio* showed an approximately normal frequency distribution that closely matched that of emergence holes (which is logical because the head capsule width equals the minimum required diameter of the emergence hole): mean ± SD = 3.3 ± 0.6 mm, min – max = 1.5 – 4.5 mm).

**The form of feeding galleries.**

It was quite easy to distinguish galleries of Siricidae from those of woodboring beetles when we cut and split logs to expose feeding galleries (Fig. 2). Deeper than 1-2 cm into the wood below emergence holes, woodborer galleries were always clearly oval – not round (because the larvae have a flattened morphology). They also tended to emerge at a more oblique angle than *S. noctilio* (Siricidae instead?) and could often be associated with the large elliptical sapwood entrance holes where late instars, particularly *Monochamus* spp., had entered the xylem very obliquely from the phloem. Furthermore, the frass of *Monochamus* spp. is macroscopically fibrous (many fibers of at least 1 mm in length), and tends to easily fall out of the feeding galleries in contrast to the fine, tightly packed frass of Siricidae. Buprestid larvae, which were also present in low numbers, chew nearly as finely as Siricidae spp., but their feeding galleries are clearly oval within the wood, and their exit holes are seldom or never as perfectly round as Siricidae (typically D-shaped). In field surveys, we were generally able to distinguish suspiciously round woodborer holes from those of Siricidae by using a hatchet to expose the gallery 5-10 mm into the wood, where woodborer galleries generally become recognizably oval (Fig. 4).

**The spatial patterning of emergence holes.**

A further diagnostic feature of *S. noctilio* is that emergence holes tend to be aggregated on particular trees and further clumped within trees (presumably because the female adults tend to oviposit repeatedly in the same area when they find a suitable host tree). Woodborers, in contrast, tend to be distributed relatively evenly across recent dead pine trees within stands (citation?) and distributed throughout the bole of the tree (citation?). From our dissections of trap trees, 9 of 15 logs that contained *S. noctilio* had more than 5 emergence holes, and all had at least one 2 - 3 mm in diameter. Thus, a pine tree with clumps of five or more circular holes within a 55 cm log section (?), with one or more of
those holes being 2 - 3 mm in diameter, is a good candidate for having contained S. noctilio larvae. Such patterns generally turned up quickly in ground surveys within regions known to harbor S. noctilio, and were detected only with considerable searching in the eastern Adirondack region, where S. noctilio has not yet been detected. The holes remain evident for many years, even after the dead, previously infested, trees have fallen (Fig. 5).

Within infested stands and regions, S. noctilio displayed a highly aggregated dispersion among trees. The frequency distributions of carefully measured emergences from scots and red pine were well approximated by a gamma distribution, with frequent occurrences of trees that produced several-fold more S. noctilio than the median tree (Fig. 6, mid and lower). The patterning of Sirex-like emergence holes in dead trees in Finger Lakes National Forest (known to be infested with S. noctilio based on adult specimens and frequent occurrence of resin drippings from attacks) revealed a very similar highly aggregated pattern of tree-specific emergence (Fig. 6, upper). Thus, a further diagnostic of occupancy by S. noctilio is that a sample of 20 or 30 recently dead hard pine trees is quite likely to reveal at least one tree with conspicuously high abundance of Sirex-like emergence holes. From the tree population that we sampled with binoculars, a random sample of > 20 dead pines was > 95% certain to produce at least one tree with ≥ 10 Sirex-like emergence holes that were evident by non-invasive examination of one face of the bole (4x the median abundance of 2.5) (Fig. 6, upper inset). In this, and other populations of dead hard pines, we found the dispersion of emergence holes from woodboring beetles to be strikingly different in their uniformity (more closely approximating a normal than a gamma distribution). When the sampling involved trap trees, even smaller samples (< 15 trees) were sufficient to make it almost certain that the signal of occasional trees with conspicuously high abundance would be detected (Fig. 6, middle and lower insets), but of course it is more laborious to create trap trees and to capture the emerging insects). Corley et al. (2007) similarly reported highly aggregated dispersion of S. noctilio in Argentina.

As has been described previously for S. noctilio (Dodds et al. 2010), emergence holes were generally in the mid-bole – most from 2 m to the base of the live crown -- and rarely in unusually large branches. They appeared to be more likely to occur within or near branch whorls than expected by chance.

**Distinguishing the European woodwasp from native woodwasps.**

Based on even careful examination of emergence holes and feeding galleries, it is not presently possible to positively distinguish the European woodwasp, Sirex noctilio, from native species of Siricidae (e.g., Sirex nigricornis Fabricius, S. edwardsii Brullé, Urocerus albicornis Fabricius, and/or U. cressoni Norton). The native species seem to be very rare compared to S. noctilio (Long et al. 2009 reported < 1: 20 S. nigricornis: S. noctilio and < 1: 60 for S. edwardsii). Distinguishing woodwasp species is straightforward if the adults can be examined directly (Schiff et al. 2006), but adults are difficult to capture and only present for a few weeks per year. Lacking specimens, an additional diagnostic is the presence of resin on the outer bark of trees (Fig. 8). Careful examination of dying or recently dead trees that have been attacked by S. noctilio can sometimes reveal the presence of resin on the outer bark, even a year or more after the time of oviposition by woodwasp adults. The presence of resin, in combination with aggregations of appropriately sized Sirex-like emergence holes, seems to be a quite reliable indicator of the presence of S. noctilio. A related symptom is that S. noctilio are more likely to be found emerging from trees that are not dead yet (retain partial or full green crowns). Unfortunately, the failure to detect resin on trees is much less definitive.
because: (1) \textit{S. noctilio} can be present without attacking live trees, (2) the resin can disappear and, (3) the resin can be difficult to detect even when present.

Patterns of host use can provide additional information. In north central New York State, more \textit{S. noctilio} emerged from a larger proportion of scots pine trap trees than red pine trap trees (Fig. 6, Zylstra et al. 2010). Dodds et al. (2010) similarly reported higher incidence of \textit{S. noctilio} in scots pine relative to red pine. A preference of \textit{S. noctilio} for scots pine is not surprising because this is a frequent host in their native European habitats (Dajoz 1980) while they have no evolutionary history with red pine. We guess that all or most of the \textit{Sirex} spp. that we detected in the Adirondacks were native species rather than \textit{S. noctilio} partly because Siricidae emergence holes were very rare within apparently suitable pine stands and seldom displayed conspicuous aggregations, and partly because we could find no signs of resin drops, but also because they were found almost exclusively in red pine even when weak and dying scots pine were in the immediate vicinity. Further studies are needed to understand the comparative host preferences of \textit{S. noctilio} with species of Siricidae native to North America.

A protocol for determining presence and absence of \textit{S. noctilio}.

The above information suggests a protocol to structure ground surveys for the presence of \textit{S. noctilio} (Fig. 9). The process begins by locating stands of hard pine (e.g., red or scots pine) that contain dead and/or dying trees (e.g., Fig. 7). Efforts should first be focused on smaller diameter suppressed trees that have poor crown development (Dodds et al. 2010). Examination of the surface of tree boles can quickly indicate the absence of \textit{S. noctilio}. Many insect species feed in the phloem whereas \textit{Sirex} larvae feed exclusively within the xylem. So holes that do not come from the wood itself cannot have been produced by \textit{Sirex}. For this reason, it is easier to visually scan for Siricidae emergence holes in sections of tree trunk where the bark has fallen away or is removed by the observer. If there are no Siricidae-like holes in the wood (see below), then there were no siricids that emerged from at least that section of the tree. If there are circular emergence holes from the xylem of the mid-bole, some of which are 1.5 to 3 mm in diameter, and especially if there are sometimes aggregations of holes, then it is probable that the stand has harbored \textit{S. noctilio}, and further examination is warranted. If sections can be cut or split, and the internal galleries (and frass) match that of Siricidae (Figs. 1 and 3), then it is almost certain that the log contained \textit{Sirex} spp. If there are resin drops or drippings as in Fig. 8, and/or if some emergence holes are in trees that are not yet dead, then it is very probable that the stand harbors \textit{S. noctilio}. It is not possible with any known sampling techniques to be sure that a region lacks \textit{S. noctilio}, especially since they must be rare when initially extending their range into a new area. However, with populations such as currently exist in central New York and northern Pennsylvania, examination of any 5 or 10 stands of hard pine with numerous dead and dying trees was almost sure to reveal \textit{Sirex}-like emergence holes. Our sense is that the absence of \textit{Sirex}-like emergence holes in a survey of at least a few recently dead trees within 20 – 25 such stands in an area would quite reliably indicate the absence of an established \textit{S. noctilio} population. With the protocol that we suggest, such a survey could be accomplished with just a few days of work by one or two people, and would not necessarily require more than binoculars and a hatchet.

Of course definitive verification of the presence of \textit{S. noctilio} within a new region requires voucher specimens of adults, especially because not much is known about the abundance and distribution of native Siricidae that produce very similar galleries and emergence holes. With perseverance and luck, it may be possible to capture adults by hand, net, or traps during their flight season (July and August in New York state) if you are in a susceptible stand. Another option is to locate one or more currently infested
trees and capture adults as they emerge (most easily by putting infested sections in emergence cans). Sections of bole that contain Sirex larvae can generally be identified by the presence of galleries in ends that have been cut with a chain saw (Fig. 1, upper right). Also, such sections of log tend to be noticeably lighter than adjacent uncolonized sections. Under some circumstances it may be desirable and practical to create and monitor trap trees for sampling a local community of woodboring insects, including Sirex spp. (Zylstra et al. 2010). An alternative possible tactic for identifying Sirex spp. is to isolate and identify the associated fungus (e.g., by sequencing the ITS and / or IGS regions of ribosomal DNA; Slippers et al. 2000, Bergeron et al. 2008). S. noctilio is thought to be generally associated with the fungus Amylostereum areolatum (Wilson et al. 2009) whereas Sirex spp. native to North America tend to associated with A. chailletii although this is apparently not always true (Nielsen et al. 2009). Where S. noctilio is discovered, examination of older logs can provide information about the duration of occupation. Hard pines in the region of interest typically occur in even-aged stands so it can be relatively simple to determine the time of death (by comparing ring counts of dead trees to the age of live trees). S. noctilio typically colonizes trees as they are dying, so determining the time of death indicates the time when larvae occupied the tree. At present it is not possible to identify the species of Sirex that produced an older feeding gallery, but it may become possible to identify the species of Amylostereum (and therefore infer the species of Sirex) with molecular techniques. In the meantime, the frequency of older Sirex-like emergence holes will still provide a clue because the native Sirex spp. can be sufficiently rare (Long et al. 2009) that we can expect colonization of an area by S. noctilio to be associated with a notable increase in the frequency of Sirex emergence holes.

To aid in mapping and monitoring the distribution of S. noctilio in North America, we encourage reporting positive and negative results of surveys such as described here to a local forest health professional or the authors.

Acknowledgments

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Fig. 1. Emergence holes of *Sirex noctilio* (left) are almost perfectly circular and usually exit the xylem perpendicular to the grain. The feeding galleries within are tightly packed with fine, uniformly ground, lightly colored frass (upper right: exposed in cross-section with a chainsaw; lower right: exposed via splitting with a late instar larvae present).
Fig. 2. Comparison of the size of emergence holes produced by woodboring beetles (≈ 78:22 *Monochamus carolinensis: M. scutellatus*) relative to *Sirex noctilio*. 
Fig. 3. Images from splitting infested logs. Upper shows nearby feeding galleries of *Sirex noctilio* (lower, packed with uncolored frass) and a woodboring beetle, probably *Monochamus* sp. (fusiform shape, sometimes stained and with frass that falls out easily). Lower images show close-ups of feeding galleries from woodboring beetles.
Fig. 4. A red pine log with circular emergence holes that on the surface (upper image) resembled those of *Sirex* spp., but that were revealed to have been produced by woodboring beetles after exposure of the subsurface galleries with a hatchet (lower image). Image from site 15.
Fig. 5. Examples of *Sirex*-like emergence holes in dead trees and logs. Subsequent exposure with a hatchet showed that the internal galleries retained the circular shape characteristic of *Sirex* spp. Also diagnostic of *S. noctilio* is the clustering of emergence holes and their variable sizes (≈ 2 - 6 mm). Locations, clockwise from upper left: sites 28, 78, and 57.
Fig. 6. Upper: frequency distribution of Sirex-like emergence holes in a stand of scots pine inhabited by S. noctilio (Fig. 7). Middle and lower: frequency distributions of emerging S. noctilio from trap trees of scots and red pine (Zylstra et al. 2010). Solid lines in main figures show fits to a gamma distribution. Insets show the probability, based on the empirical distributions, of finding at least one tree with conspicuously high abundance as a function of number of trees sampled.
Fig. 7. Scots pine stand in Schuyler County, NY (site 309) inhabited by *S. noctilio* (source of data in Fig. 6, upper). Such stands with numerous dead and dying trees are likely habitat for *S. noctilio* if *S. noctilio* is present in the area.
Fig. 8. Resin on the bole of pine trees from attacks by *S. noctilio*. Resin tends to crystallize in the form of droplets on red pine but drippings on scots and maritime pine. *S. noctilio* is apparently the only species of *Sirex* that regularly attacks living trees. Therefore the presence of resin drops or drips is a diagnostic for distinguishing the presence of *S. noctilio* from other *Sirex* spp. Images from sites 132, 302, and Galicia, Spain (left to right).
Some recently dead pines with circular insect emergence holes from xylem 1.5 – 6 mm diameter (= SLH, Sirex-like holes)?

No

Some SLHs 1.5-3.5 mm?

Yes

No

Some conspicuous aggregations of SLHs within trees?

Yes

No

Sirex spp. probable

Sirex-like frass and feeding galleries within logs?

Yes

No

Sirex-like resin drips on some pines and/or some SLHs in trees with crowns green or partially so.

Sirex spp. probably present, but not necessarily S. noctilio. Further investigation warranted.

Yes

S. noctilio probably present. Further investigation warranted.

Fig. 9. A protocol for rapid initial indications of the presence or absence of Sirex spp in general, and S. noctilio in particular, by examination of dead and dying pine trees.
**Appendix.** Stands of hard pine inspected on foot during July 2009 for signs of *Sirex* spp. All stands contained recently dead pines that were inspected for *Sirex*-like emergence holes. When *Sirex*-like emergence holes were detected, we further searched for resin drops or drippings (characteristic of *S. noctilio* attacks) on declining.

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<td><em>Sirex</em> spp.</td>
</tr>
<tr>
<td>31</td>
<td>NY: Adirondacks</td>
<td>44.22</td>
<td>74.31</td>
<td>Red</td>
<td>Yes</td>
<td>No</td>
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<td>44.43</td>
<td>74.18</td>
<td>Red/Scots</td>
<td>Yes</td>
<td>No</td>
<td><em>Sirex</em> spp.</td>
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<td>120</td>
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<td>76.79</td>
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