Supplement for: Porter S.D., Plowes, R.M. 2017. Biology and rearing of the decapitating fly *Pseudacteon bifidus* (Diptera: Phoridae) a parasitoid of tropical fire ants. Florida Entomologist ??:??

Rearing *Pseudacteon bifidus*: Notes, Protocols, Quality Control, and Photos

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We used mostly the same procedures and equipment for rearing *P. bifidus* on *Solenopsis geminata* as we used for rearing *P. cultellatus* on *Solenopsis invicta* (Porter et al. 2013). Below are modifications for rearing *P. bifidus*:

Changes for Rearing P. bifidus

1. In order to reduce rearing costs, we reared separate generations rather than overlapping generations. This reduced production, but also probably reduced rearing costs by at least 30% because host attacks and other time consuming tasks did not occur continuously.

2. We used workers from the field without attempting to modify their size ratios by sieving them. Sieving was not needed because the proportion of larger workers in *S. geminata* colonies is much less than for *S. invicta* colonies.

3. Because of large variability in host colony suitability, we tried to use 4 colonies for each box run (two trays with 1 g of workers from each colony – 8 total trays). Workers from colonies that were poor hosts were not reused in the next generation.

4. We did not need to worry about contamination from *P. curvatus* flies because *P. curvatus* does not successfully cycle in *S. geminata* workers.

5. We did not need to worry about SINV-3 virus infections because *S. geminata* is not susceptible to infections from this virus.

6. We added *S. invicta* brood to keep *S. geminata* workers running during fly oviposition attacks and then removed most of the *S. invicta* brood after attacks. This was done because rearing large quantities of *S. invicta* brood was much easier than rearing similar amounts of *S. geminata* brood. *Solenopsis* brood can be cross fostered between Solenopsis species until adult workers start eclosing.

7. Flies were provided with sugar wicks and water wicks in the attack box (see photos in this supplement). Access to sugar and water seemed to improve their longevity, but was probably not essential.

8. Flies were left in the attack boxes for 4-6 days or 1-2 days more than was the case for *P. cultellatus* and other *Pseudacteon* flies we have reared. This was because *P. bifidus* males emerge about a day earlier than females and because females require an extra 6-24 h before they are ready to oviposit.

9. We accelerated the development of the last several days of puparia for each generation by holding them at higher temperatures so that they would emerge earlier and fit within a 16-20 day window for oviposition attacks.

Other Notes:

Please inspect the task lists included with this supplement for more details about rearing procedures. The supplement for rearing *P. cultellatus* (Porter et al. 2013) includes photographs for supplies and procedures not included in this supplement including details about mold control on pupae trays and minimizing the build up of mites and "trash phorids" (*Megaselia* spp.).

For further information about Pseudacteon fly rearing see:

Vogt, J. T., S. D. Porter, D. A. Nordlund, and R. Smith. 2003. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biol. Control 28: 346-353.

Porter, S. D., V. Kumar, L. A. Calcaterra, J. A. Briano, and D. R. Seal. 2013. Release and establishment of the little decapitating fly *Pseudacteon cultellatus* on imported fire ants in Florida. Fla. Entomol. 96: 1567-1573. and Supplement. http://journals.fcla.edu/flaent/article/view/82794/79700 or http://www.ars.usda.gov/saa/cmave/ifahi/cultellatus

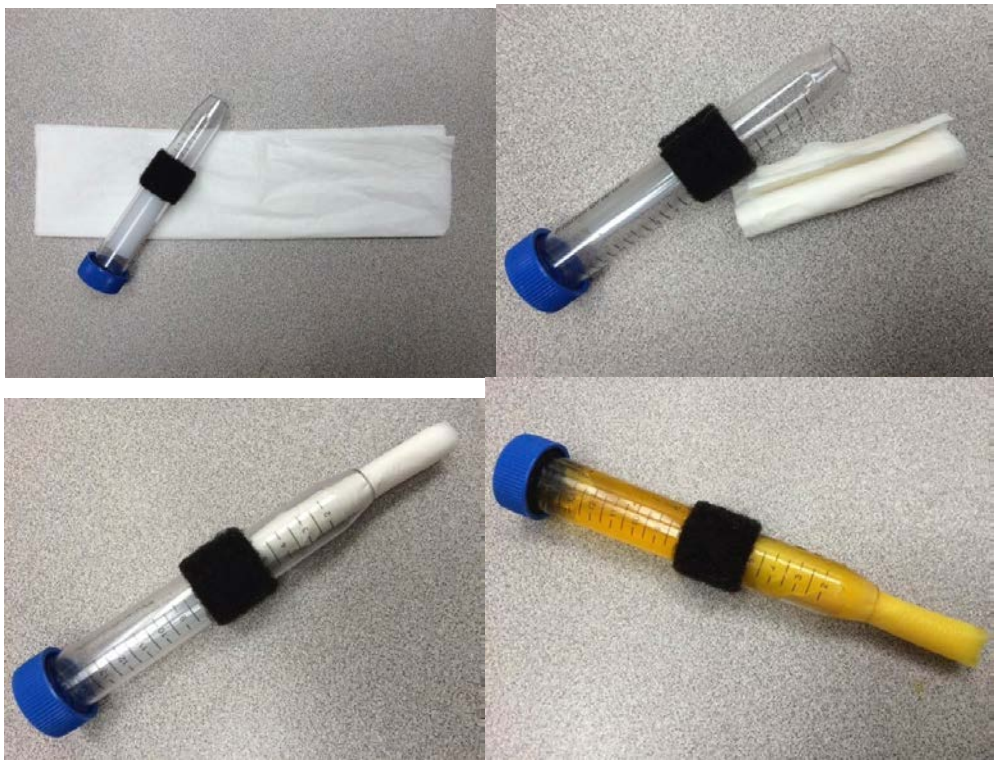
Habitat near Nueces River where *Pseudacteon bifidus* flies were collected.

Pseudacteon bifidus flies were collected using white trays with about 5 g of *Solenopsis geminata* workers and about the same amount of brood. A black trap nest with round moisture blocks was placed in each tray and moved from one end to the other whenever most of the ants and brood had taken refuge under it. This kept the ants moving so the flies could oviposit in them. The clear water tube and the yellow sugar tube were removed during fly attacks and returned afterwards.

Two automatic attack boxes used for rearing *P. bifidus* flies. Halogen lights on either end were used for heating. A vaporizer was placed in the bottom chamber under the attack chamber and a fan continuously blew humid air through the silver or black ducts into the top chamber. The vaporizer was controlled by a humidistat. Gloved sleeves allowed us to work in the boxes without flies escaping.

Refuge cups inside the attack boxes that were automatically raised and lowered every 10-12 minutes to keep the ants running back and forth while the flies attacked.

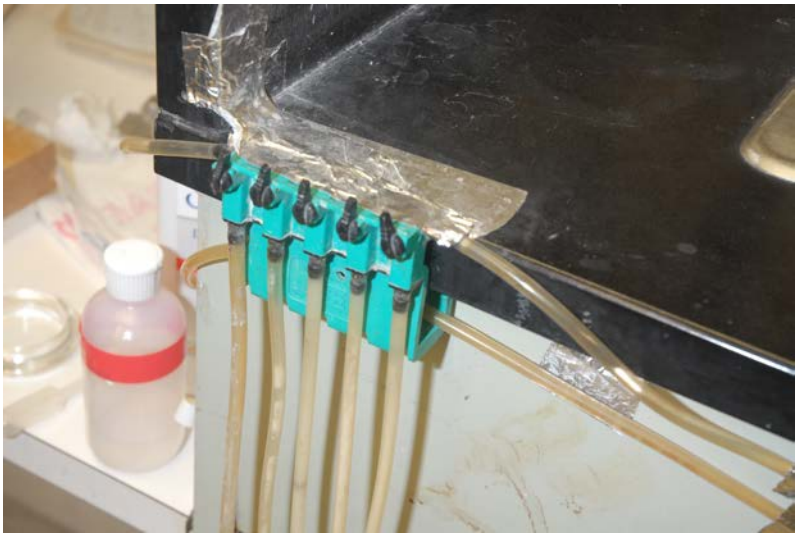
Timers on top of attack box used for controlling lights, temperature, and the pneumatic motor. Vaporizer in bottom chamber inside large tray with water.



Assembly of a sugar wick with a tubular roll of lab tissues (2 sheets, 11 by 21 cm, Kimwipes) inserted into the end of a 15 ml plastic centrifuge tube with the end cut off. The tubes were then filled with 10% sugar water (dyed yellow) and attached to the underside of the clear plastic top of the attack chamber using Velcro. Each chamber contained 4 sugar tubes and 2 water tubes. The tubes usually needed to be replaced after 4 days.

Smaller attack chamber used for rearing *Pseudacteon catarinae*. Males and females were introduced at the start of each day.

Solenopsis geminata workers were collected into 5 gal buckets and allowed to dig out over night before water was slowly dripped in to flood the ants and brood out of the dirt.



Aquarium gang valve used to control rate of water dripping into the buckets. A water pressure valve (not shown) was used to control water pressure fluctuations and a water return tube extended from the gang valve back into a sink so the valves could be closed without water pressure building up and blowing off the tubes.

Daily Tasks List for *P. bifidus* Date: _____

All day Updated: 10 August 2015

#	Task	Time	Initials	Comments
1.	**Agitate ants in attack boxes regularly** & record it on the ant trailing log	Every 0.5 - 1 hr		Timer
2.	Make supplies: *Fly Wicks*: Sugar ___ Water ___ *for Attack Trays*: Water tubes ___ Sugar tubes ___ *for #3 Boxes*: Sugar tubes ___ Moisture blocks ___ Nest tubes (with castone) ___ #3 boxes, fluoned ___ White trays, fluoned ___ Plaster trays ___ Line trays for weekend ___	As needed		
3.	Recondition big attack boxes			after each cycle
4/	Bleach plaster trays and moisture blocks after use			
5.	Collect *S. geminata* colonies in field	As needed		
Morning				
1.	Check temp.; RH, and lifters. Add water in humidifiers, if needed & sign off log sheet.			
2.	Check Fly Wicks on attack box lids. Change Monday and Friday ***or as needed***	<9:30AM		
3.	Check for notes on previous task list			
4.	Set up attack boxes, if needed Remember to write down the "attack date"			
5.	Estimate number of pupae on tray			
6.	Pull dead 1) Pull ants 2) Water blocks (damped them off, if excess of water) 3) *Change sugar tubes on **Mondays*** 4) Tray parasitized ants & place in Holding box #1 (Chamber #1)	< Noon		
7.	Move 5 day old pupae tray from Holding box #1 to Holding box #2 in main lab room **Note:** Check pupae tray set up date			
8.	Emergence rates for old pupae trays, after removed from attack box (keep up to date to spot problems)			
Afternoon				
2.	Feed ant colonies in chamber #2 **Note:** M-W-F			
3.	Breakdown attack boxes, if needed. Remember to write down the date			
4.	Put ants to bed (place in # 3 boxes), if broken down attack boxes. Each #3 box should contain: 1) Broodless ants 2) 3 moisture blocks (1 smaller block under metal cup) 3) 1 - 16 x 125 mm nest tube (with cotton + castone) 4) 1 – 10 x 75 mm sugar tube **Note:** Separate by colony or type of brood. Combine NO more than 2 attack box trays per #3 box. If 3 trays, combine them and split into two #3 boxes.			
5.	Water moisture trays in Holding box #1 **Note:** Mondays & Thursdays	>2:00PM		
6.	Bleach pupae trays in Holding box #2 (1% bleach) and water moisture trays. **Note:** Mondays & Thursdays	>2:00PM		
7.	Move pupae new trays into attack boxes, as needed	>2:00PM		
8.	Transfer and check pupae trays inside attack boxes for emergence. Move spent trays into reach-in holding box after 3 days	every 3 days		
9.	Water lightly trays inside attack boxes	>2:00PM		
10.	Empty trash, as needed, and carry bags into hall			

Daily Tasks List for *P. bifidus -Weekend* Date: _____

Updated: 10 Oct. 2015

#	Task	Time	Initials	Comments
1.	Agitate ants in attack boxes regularly & record it on the ant trailing log	Every 0.5 - 1 hr		Timer
2.	Check temp.; RH, and lifters. Add water in humidifiers, if needed & sign off log sheet.			
3.	Check for notes on previous task list			
4.	Check sugar + water tubes on attack box lids. Change if needed.	early		
5.	Pull dead 1) Pull ants 2) Water blocks (damped them off, if excess of water) 3) Tray parasitized ants & place in Holding box #1 (Chamber #1)			
6.	Move 5 day old pupae tray from Holding box #1 to Holding box #2 in main lab room **Note:** Check pupae tray set up date			
7.	Move pupae trays into attack boxes if flies are emerging in holding box 2.	early		

Special Instructions:

Notes:

Weekly Quality Control

Date_____

Rearing Estimates
_____Production estimates current and stable?
_____Emergence estimates 80-90%?
_____Rearing problems, shipments, etc. recorded?

Feeding Stock Colonies and Other Ant Colonies
_____Is brood production sufficient for needs?
_____Crickets adjusted to colony brood levels?
_____Sugar wad cups watered alternately?
_____Sugar wads replaced when sugar is exhausted or moldy?
_____Nests neither wet nor dry?
_____Sufficient nests for all workers?
_____Water tubes contain water?
_____Trays need to be cleaned?
_____Non-producing colonies disposed of?
_____Temperature **as posted**?
_____Is fluon still effective on trays?
_____Are floors clean?
_____Using good hygiene to contain viruses?

Collecting Dead
_____Are sugar Tubes replaced in all boxes once a week?
_____Dead ants thoroughly removed from boxes?
_____Dead ants dumped from collecting flask every 4 boxes?
_____Moisture blocks properly watered?
_____ Moisture blocks positioned in center of the boxes not close to edges, can cause floun breakdown.
_____Metal cup over metal tape?
_____Colony lids powered?
_____Dead ants evenly spread over plaster tray?
_____Plaster in trays damp but not wet to the touch?
_____Live ants removed (use separation technique if more than a dozen)?
_____ Copper tubes in vacuum corks cleaned after every use? Screens clear?
_____Orphan box no more than 1 month old?
_____8 day calculations correct?

General Lab tasks
_____Trash dumped in kill buckets?
_____Fluon regularly agitated and sheet being initialed and dated?
_____ Bottles labeled properly, ie. Water, bleach, alcohol?
_____ Floors swept weekly, especially in Quarantine

Big Rearing Room Environment
_____Rearing room temperatures as posted?
_____A/C vent in the ceiling adjusted appropriately?
_____Light timer functioning correctly?

Holding Box and Moving Pupae Trays
_____Trays watered Monday and Thursday and sheet initialed?
_____Bleach water freshly made (1%) and only used on trays 5 days or older?
_____ Holding boxes closed properly to prevent drying out pupae trays?
_____Trays are kept in chronological order?
_____Boxes wiped out every couple weeks?

Big Attack Boxes and Room
_____Ant trailing sheet being used?
_____Lids moving slowly and evenly?
_____Check lifter lids for broken strings?
_____Are honey and water tubes on lid fresh?
_____Are all timers set to the correct time on attack box and room?
_____Are the timers working properly (program and auto-on/off on selected)?
_____Halogens working?
_____Humidifier working properly?
_____Water for humidifier replaced monthly and humidifier cleaned (see check sheet)?
_____Check that humidifier light is working?
_____Box vacuumed out and checked for spiders and stray ants regularly?
_____Plexiglas lid cleaned as needed?
_____ All environmental conditions in the attack box appropriate? Temperature 26.5°-27.5°C? Humidity 80-90%?
_____ Humidifier water checksheet updated daily?
_____ Water in bottom ≤ 90°F for safety off.
_____ Fluoned white attack trays removed and cleaned when needed?

Loggers
_____Have the loggers been downloaded monthly?
_____Rearing rooms?
_____Attack boxes?

Autoclave
____ Trash autoclaved weekly

Small Attack Boxes

____ Plaster clean no mold?
_____ Plaster flush with side of box?
_____ Lifters properly working?
_____ Lifter cups even so ants can't crawl up them?
_____ Fresh water tube in back port, and inserted properly?
_____ Gaskets checked regularly?
_____ Fluon still intact?
_____ Penny sheets cleaned?
_____ Flower cups fluon intact?
_____ Motors still in working order.

Supplies

_____ Sugar tubes for attack box in fridge
_____ Water tubes for attack box in fridge (2 wks)
_____ Sugar tubes for parasitized workers in fridge (2 wks)
_____ #3 boxes fluoned for parasitized ants (10 Bldg 26, 20 Quarantine)
_____ pupae trays (
_____ Moisture blocks (40 Quarantine, 40 Bldg 26)
_____ Water and Honey wick tubes for flies

_____ White trays for attack boxes (8 Q + 8 Bldg 26)
_____ Large grey fluoned nest trays for *geminata* and Imported Fire ants.
_____ Small black boxes fluoned for small colonies
_____ Nest Cells for black boxes
_____
_____
_____

Water Pupae Trays (1% bleach = 5 days or older; water for blank trays; NO water for first 4 days)

2014			
Mondays	Initials	Thursdays	Initials
7-Jul		10-Jul	
14-Jul		17-Jul	
21-Jul		24-Jul	
28-Jul		31-Jul	
4-Aug		7-Aug	
11-Aug		14-Aug	
18-Aug		21-Aug	
25-Aug		28-Aug	
1-Sep		4-Sep	
8-Sep		11-Sep	
15-Sep		18-Sep	
22-Sep		25-Sep	
29-Sep		2-Oct	
6-Oct		9-Oct	
13-Oct		16-Oct	
20-Oct		23-Oct	
27-Oct		30-Oct	
3-Nov		6-Nov	
10-Nov		13-Nov	
17-Nov		20-Nov	
24-Nov		27-Nov	
1-Dec		4-Dec	
8-Dec		11-Dec	
15-Dec		18-Dec	
22-Dec		25-Dec	
29-Dec		1-Jan	

2015			
Mondays	Initials	Thursdays	Initials
5-Jan		8-Jan	
12-Jan		15-Jan	
19-Jan		22-Jan	
26-Jan		29-Jan	
2-Feb		5-Feb	
9-Feb		12-Feb	
16-Feb		19-Feb	
23-Feb		26-Feb	
2-Mar		5-Mar	
9-Mar		12-Mar	
16-Mar		19-Mar	
23-Mar		26-Mar	
30-Mar		2-Apr	
6-Apr		9-Apr	
13-Apr		16-Apr	
20-Apr		23-Apr	
27-Apr		30-Apr	
4-May		7-May	
11-May		14-May	
18-May		21-May	
25-May		28-May	
1-Jun		4-Jun	
8-Jun		11-Jun	
15-Jun		18-Jun	
22-Jun		25-Jun	
29-Jun		2-Jul	

2015			
Mondays	Initials	Thursdays	Initials
6-Jul		9-Jul	
13-Jul		16-Jul	
20-Jul		23-Jul	
27-Jul		30-Jul	
3-Aug		6-Aug	
10-Aug		13-Aug	
17-Aug		20-Aug	
24-Aug		27-Aug	
31-Aug		3-Sep	
7-Sep		10-Sep	
14-Sep		17-Sep	
21-Sep		24-Sep	
28-Sep		1-Oct	
5-Oct		8-Oct	
12-Oct		15-Oct	
19-Oct		22-Oct	
26-Oct		29-Oct	
2-Nov		5-Nov	
9-Nov		12-Nov	
16-Nov		19-Nov	
23-Nov		26-Nov	
30-Nov		3-Dec	
7-Dec		10-Dec	
14-Dec		17-Dec	
21-Dec		24-Dec	
28-Dec		31-Dec	

Humidifier Water - Daily Check Sheet

Date	Initials	Date	Initials	Date	Initials	Date	Initials	Date	Initials	Date	Initials	Date	Initials
27-Jul-14		03-Sep-14		11-Oct-14		18-Nov-14		26-Dec-14		02-Feb-15		12-Mar-15	
28-Jul-14		04-Sep-14		12-Oct-14		19-Nov-14		27-Dec-14		03-Feb-15		13-Mar-15	
29-Jul-14		05-Sep-14		13-Oct-14		20-Nov-14		28-Dec-14		04-Feb-15		14-Mar-15	
30-Jul-14		06-Sep-14		14-Oct-14		21-Nov-14		29-Dec-14		05-Feb-15		15-Mar-15	
31-Jul-14		07-Sep-14		15-Oct-14		22-Nov-14		30-Dec-14		06-Feb-15		16-Mar-15	
01-Aug-14		08-Sep-14		16-Oct-14		23-Nov-14		31-Dec-14		07-Feb-15		17-Mar-15	
02-Aug-14		09-Sep-14		17-Oct-14		24-Nov-14		01-Jan-15		08-Feb-15		18-Mar-15	
03-Aug-14		10-Sep-14		18-Oct-14		25-Nov-14		02-Jan-15		09-Feb-15		19-Mar-15	
04-Aug-14		11-Sep-14		19-Oct-14		26-Nov-14		03-Jan-15		10-Feb-15		20-Mar-15	
05-Aug-14		12-Sep-14		20-Oct-14		27-Nov-14		04-Jan-15		11-Feb-15		21-Mar-15	
06-Aug-14		13-Sep-14		21-Oct-14		28-Nov-14		05-Jan-15		12-Feb-15		22-Mar-15	
07-Aug-14		14-Sep-14		22-Oct-14		29-Nov-14		06-Jan-15		13-Feb-15		23-Mar-15	
08-Aug-14		15-Sep-14		23-Oct-14		30-Nov-14		07-Jan-15		14-Feb-15		24-Mar-15	
09-Aug-14		16-Sep-14		24-Oct-14		01-Dec-14		08-Jan-15		15-Feb-15		25-Mar-15	
10-Aug-14		17-Sep-14		25-Oct-14		02-Dec-14		09-Jan-15		16-Feb-15		26-Mar-15	
11-Aug-14		18-Sep-14		26-Oct-14		03-Dec-14		10-Jan-15		17-Feb-15		27-Mar-15	
12-Aug-14		19-Sep-14		27-Oct-14		04-Dec-14		11-Jan-15		18-Feb-15		28-Mar-15	
13-Aug-14		20-Sep-14		28-Oct-14		05-Dec-14		12-Jan-15		19-Feb-15		29-Mar-15	
14-Aug-14		21-Sep-14		29-Oct-14		06-Dec-14		13-Jan-15		20-Feb-15		30-Mar-15	
15-Aug-14		22-Sep-14		30-Oct-14		07-Dec-14		14-Jan-15		21-Feb-15		31-Mar-15	
16-Aug-14		23-Sep-14		31-Oct-14		08-Dec-14		15-Jan-15		22-Feb-15		01-Apr-15	
17-Aug-14		24-Sep-14		01-Nov-14		09-Dec-14		16-Jan-15		23-Feb-15		02-Apr-15	
18-Aug-14		25-Sep-14		02-Nov-14		10-Dec-14		17-Jan-15		24-Feb-15		03-Apr-15	
19-Aug-14		26-Sep-14		03-Nov-14		11-Dec-14		18-Jan-15		25-Feb-15		04-Apr-15	
20-Aug-14		27-Sep-14		04-Nov-14		12-Dec-14		19-Jan-15		26-Feb-15		05-Apr-15	
21-Aug-14		28-Sep-14		05-Nov-14		13-Dec-14		20-Jan-15		27-Feb-15		06-Apr-15	
22-Aug-14		29-Sep-14		06-Nov-14		14-Dec-14		21-Jan-15		28-Feb-15		07-Apr-15	
23-Aug-14		30-Sep-14		07-Nov-14		15-Dec-14		22-Jan-15		01-Mar-15		08-Apr-15	
24-Aug-14		01-Oct-14		08-Nov-14		16-Dec-14		23-Jan-15		02-Mar-15		09-Apr-15	
25-Aug-14		02-Oct-14		09-Nov-14		17-Dec-14		24-Jan-15		03-Mar-15		10-Apr-15	
26-Aug-14		03-Oct-14		10-Nov-14		18-Dec-14		25-Jan-15		04-Mar-15		11-Apr-15	
27-Aug-14		04-Oct-14		11-Nov-14		19-Dec-14		26-Jan-15		05-Mar-15		12-Apr-15	
28-Aug-14		05-Oct-14		12-Nov-14		20-Dec-14		27-Jan-15		06-Mar-15		13-Apr-15	
29-Aug-14		06-Oct-14		13-Nov-14		21-Dec-14		28-Jan-15		07-Mar-15		14-Apr-15	
30-Aug-14		07-Oct-14		14-Nov-14		22-Dec-14		29-Jan-15		08-Mar-15		15-Apr-15	
31-Aug-14		08-Oct-14		15-Nov-14		23-Dec-14		30-Jan-15		09-Mar-15		16-Apr-15	
01-Sep-14		09-Oct-14		16-Nov-14		24-Dec-14		31-Jan-15		10-Mar-15		17-Apr-15	
02-Sep-14		10-Oct-14		17-Nov-14		25-Dec-14		01-Feb-15		11-Mar-15		18-Apr-15	

Ant Trailing Log

Date	Time	Initials	Comments

Date	Time	Initials	Comments

Date	Time	Initials	Comments