The Rice example: illustrating the first five steps for smoothing and extracting traits (SET) using growthPheno

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This example is based on the data whose analysis has been published by Al-Tamimi et al. (2016). The five steps of the method for smoothing and extracting traits (SET) described in detail in Brien et al. (2020) is illustrated for this data using `growthPhen0` (Brien, 2025g), a package for the R statistical computing environment (R Core Team, 2025).

Initialize

Step 1: Import, select and derive longitudinal data

Step 1(a): Import the data

```
data(RiceRaw.dat)
```

Step 1(b): Organize the data

Here the imaging variables are selected and covariates and factors added to produce `longi.dat`.

```
longi.dat <- prepImageData(data=RiceRaw.dat, smarthouse.lev=c("NE","NW"))

longi.dat <- designFactors(data = longi.dat, insertName = "Reps",
                           nzones = 3, designfactorMethod="StandardOrder")

#'## Particular edits to longi.dat - add Days after treatment (xDAT)
longi.dat$xDAT <- longi.dat$xDAP - 29
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag,DAP), ])
```

Step 1(c): Derive longitudinal traits that result in a value for each observation

```
# Set responses
responses.image <- c("PSA")
responses.smooth <- paste0("s", responses.image)
```

```r
# Form growth rates for each observation of a subset of responses by differencing
longi.dat <- byIndv4Times_GRsDiff(longi.dat, responses = responses.image,
                                  times = "DAP",
                                  which.rates = c("AGR","RGR"))

# Form PSA.WUI
longi.dat <- within(longi.dat,
                    PSA.WUI <- WUI(PSA.AGR*DAP.diffs, WU))

# Add cumulative responses
longi.dat <- within(longi.dat,
                    {
                      WU.cum <- unlist(by(WU, Snapshot.ID.Tag,
                                          cumulate, exclude.1st=TRUE))
                      WUI.cum <- PSA / WU.cum
                    })
# Check longi.dat
head(longi.dat)
```

```
##   Snapshot.ID.Tag DAP Smarthouse Lane Position xDAP Snapshot.Time.Stamp
## 1         045727-C  28         NE   13        2   28 2015-02-18 05:31:00
## 2         045727-C  30         NE   13        2   30 2015-02-20 05:23:00
## 3         045727-C  31         NE   13        2   31 2015-02-21 05:23:00
## 4         045727-C  32         NE   13        2   32 2015-02-22 05:23:00
## 5         045727-C  33         NE   13        2   33 2015-02-23 05:24:00
## 6         045727-C  34         NE   13        2   34 2015-02-24 10:15:00
##        Hour Reps Zone cZone SHZone ZLane ZMainunit Subunit cMainPosn cPosn
## 1  5.516667    1    1    -1      1     1         1       1     -10.5   -11
## 2  5.383333    1    1    -1      1     1         1       1     -10.5   -11
## 3  5.383333    1    1    -1      1     1         1       1     -10.5   -11
## 4  5.383333    1    1    -1      1     1         1       1     -10.5   -11
## 5  5.400000    1    1    -1      1     1         1       1     -10.5   -11
## 6 10.250000    1    1    -1      1     1         1       1     -10.5   -11
##   Genotype.ID Treatment.1 Weight.Before Weight.After Water.Amount  WU     PSA
## 1      121146     Control          4013         4032           22  NA  55.311
## 2      121146     Control          4062         4085           26 -30  80.130
## 3      121146     Control          4040         4085           48  45  94.788
## 4      121146     Control          4032         4086           56  53 108.613
## 5      121146     Control          4027         4086           61  59 133.677
## 6      121146     Control          4012         4086           76  74 157.847
##   PSA.SV1 PSA.SV2 PSA.TV Boundary.Points.To.PSA.Ratio.SV1
## 1  11.307  15.456 28.548                          0.563633
## 2  25.816  21.768 32.546                          0.342539
## 3  31.627  23.604 39.557                          0.346413
## 4  37.702  30.704 40.207                          0.342316
## 5  39.861  40.317 53.499                          0.376308
## 6  48.086  46.873 62.888                          0.320904
##   Boundary.Points.To.PSA.Ratio.SV2 Boundary.Points.To.PSA.Ratio.TV
## 1                         0.371442                         0.233571
## 2                         0.439085                         0.220304
## 3                         0.411922                         0.230477
## 4                         0.365262                         0.228343
## 5                         0.346876                         0.222883
```

```
## 6                         0.367504                         0.219342
##   Caliper.Length.SV1 Caliper.Length.SV2 Caliper.Length.TV Compactness.SV1
## 1            736.872            792.324            888.821       0.0491248
## 2            728.754            785.611            797.924       0.0994986
## 3            779.808            889.427            797.332       0.1061870
## 4            956.613            896.909            861.304       0.1008410
## 5           1076.500           1123.540           1202.150       0.0809829
## 6           1016.490           1235.360           1277.480       0.1033860
##   Compactness.SV2 Compactness.TV Convex.Hull.PSA.SV1 Convex.Hull.PSA.SV2
## 1       0.0815964      0.1069410             230.169              189.420
## 2       0.0683533      0.1051310             259.461              318.463
## 3       0.0658089      0.1030830             297.843              358.675
## 4       0.0733596      0.0916347             373.877              418.541
## 5       0.0796217      0.0908369             492.215              506.357
## 6       0.0621912      0.0986004             465.109              753.692
##   Convex.Hull.PSA.TV Center.Of.Mass.Y.SV1 Center.Of.Mass.Y.SV2
## 1            266.952              1822.21              1785.29
## 2            309.575              1809.31              1808.03
## 3            383.738              1815.14              1826.45
## 4            438.775              1827.60              1874.88
## 5            588.957              1823.38              1861.62
## 6            637.807              1830.35              1843.54
##   Max.Dist.Above.Horizon.Line.SV1 Max.Dist.Above.Horizon.Line.SV2 xDAT
## 1                             612                             626   -1
## 2                             637                             636    1
## 3                             591                             628    2
## 4                             650                             691    3
## 5                             599                             618    4
## 6                             695                             707    5
##   DAP.diffs PSA.AGR   PSA.RGR    PSA.WUI    WUI.cum WU.cum
## 1        NA      NA        NA         NA         NA     NA
## 2         2 12.4095 0.1853393 -0.8273000 -2.6710000    -30
## 3         1 14.6580 0.1679925  0.3257333  6.3192000     15
## 4         1 13.8250 0.1361483  0.2608491  1.5972500     68
## 5         1 25.0640 0.2076353  0.4248136  1.0525748    127
## 6         1 24.1700 0.1661998  0.3266216  0.7853085    201
```

Step 2: Exploratory analysis

Step 2(a): Fit splines to smooth the longitudinal trends in the primary traits and calculate their growth rates

The `smoothing.method` used is `direct` and `df` is set to 4. The growth rates are calculated by difference, rather than from the spline derivatives.

```
# Smooth responses and form growth rates by differences
for (response in c(responses.image, "WU"))
  longi.dat <- byIndv4Times_SplinesGRs(data = longi.dat, response = response,
                                       response.smoothed = paste0("s", response),
                                       individuals = "Snapshot.ID.Tag", times="DAP",
                                       df = 4)
```

```
## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline
## - all fitted values set to NA
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## - all fitted values set to NA
## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline
## - all fitted values set to NA

## Warning in log(PGR(x, time.diffs, lag = lag)): NaNs produced
```
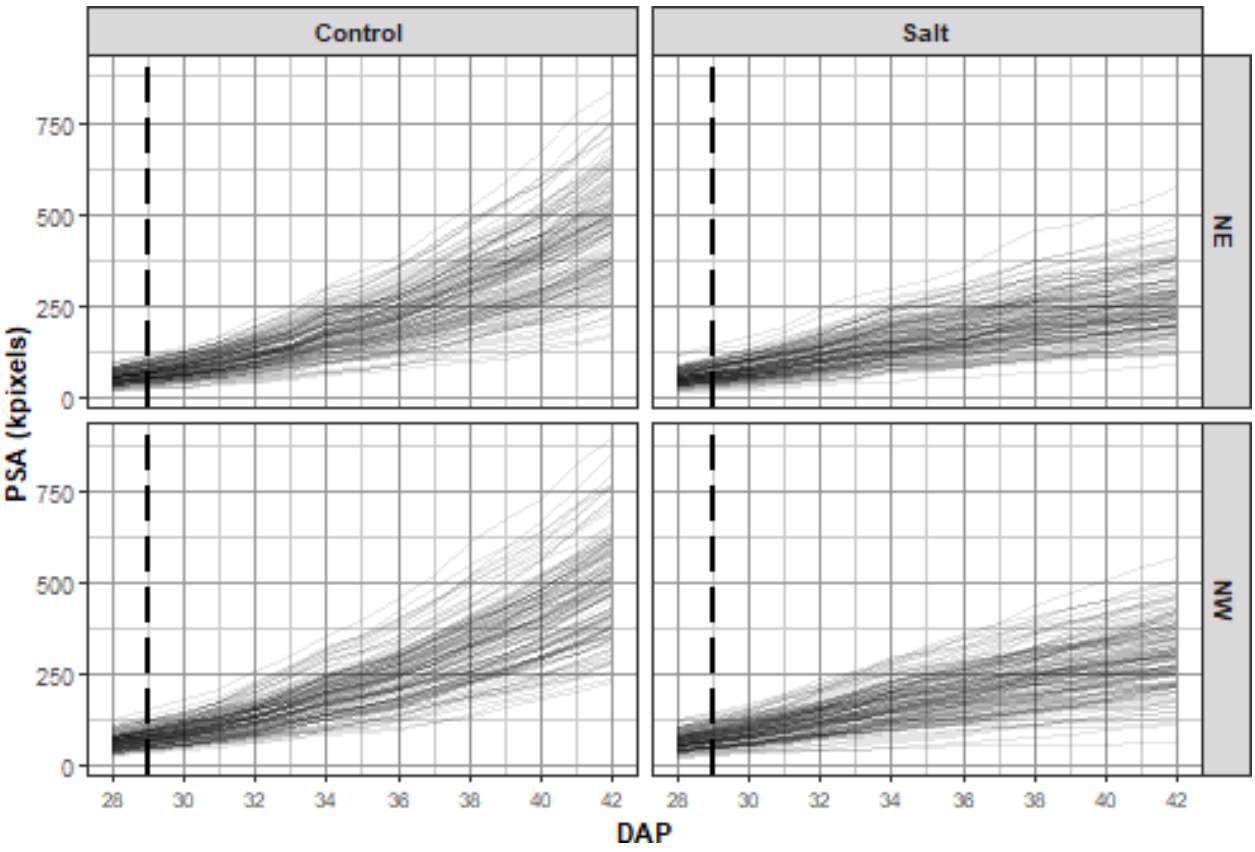

```
# Finalize longi.dat
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag, xDAP), ])
```

Step 2(b): Compare plots of unsmoothed and smoothed longitudinal data

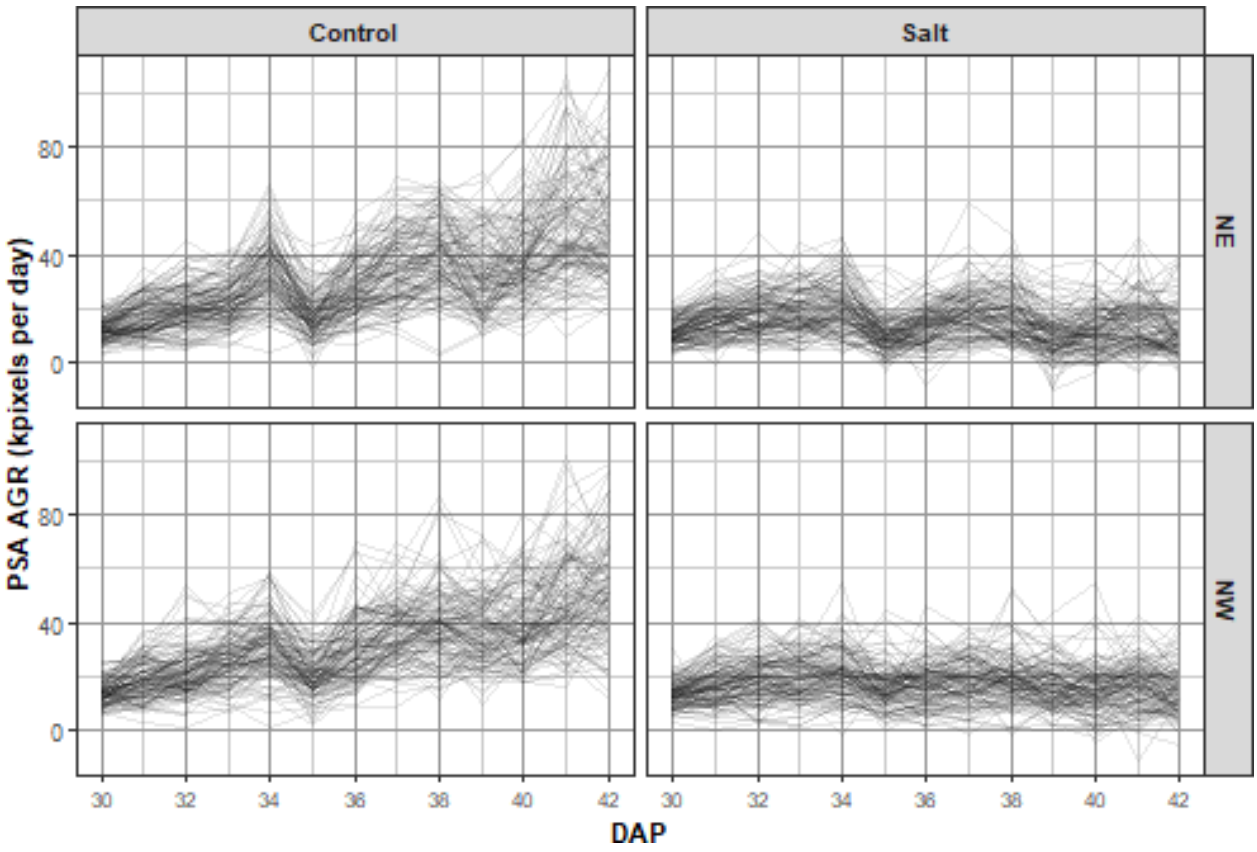
```
responses.longi <- c("PSA","PSA.AGR","PSA.RGR", "PSA.WUI")
responses.smooth.plot <- c("sPSA","sPSA.AGR","sPSA.RGR")
titles <- c("PSA (kpixels)",
            "PSA AGR (kpixels per day)", "PSA RGR (per day)",
            "PSA WUI (kpixels per mL)")
titles.smooth<-paste0("s", titles)
nresp <- length(responses.longi)
```

Plot unsmoothed profiles for all longitudinal responses

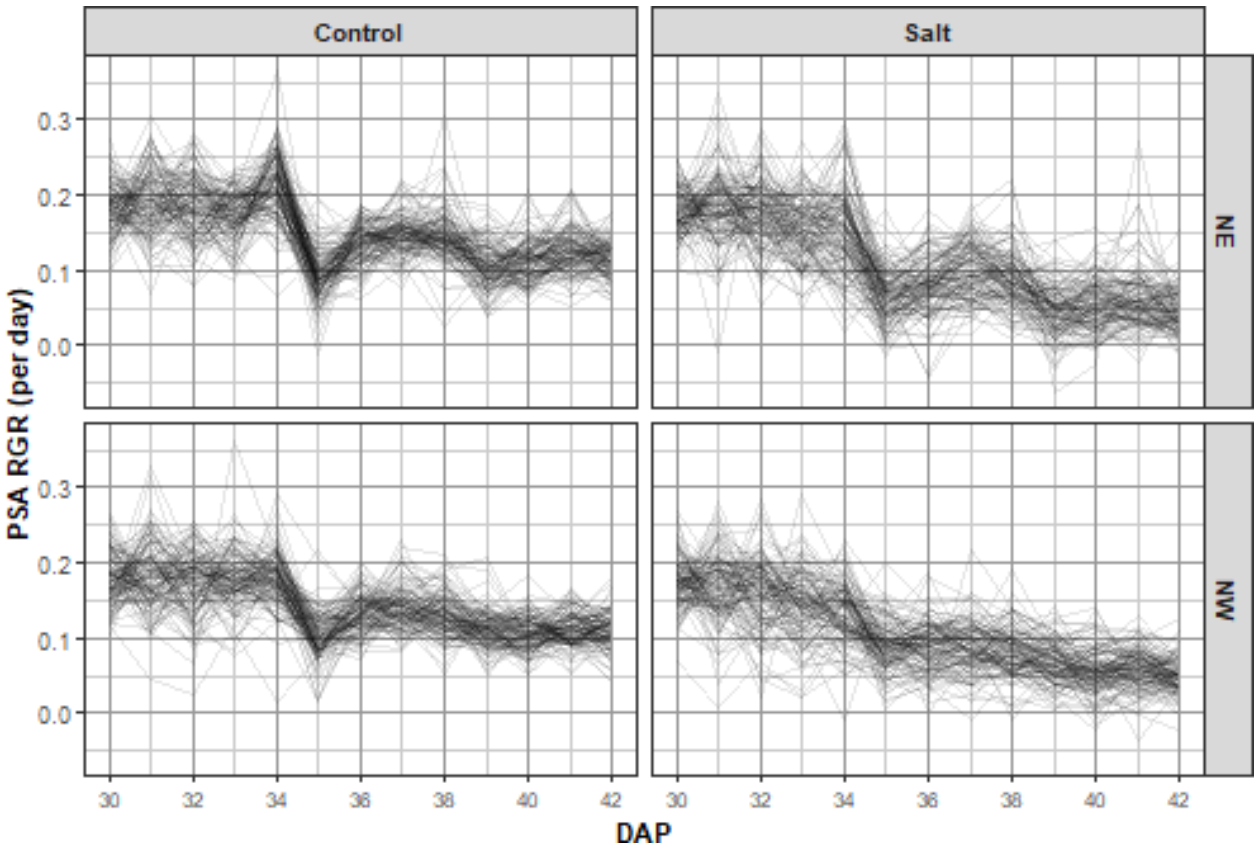
```
for (k in 1:nresp)
{
  plt <- plotProfiles(data = longi.dat, response = responses.longi[k],
                      y.title = titles[k], times = "DAP",
                      facet.x = "Treatment.1", facet.y = "Smarthouse",
                      breaks.spacing.x = 2,
                      printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1)
  print(plt)
}
```



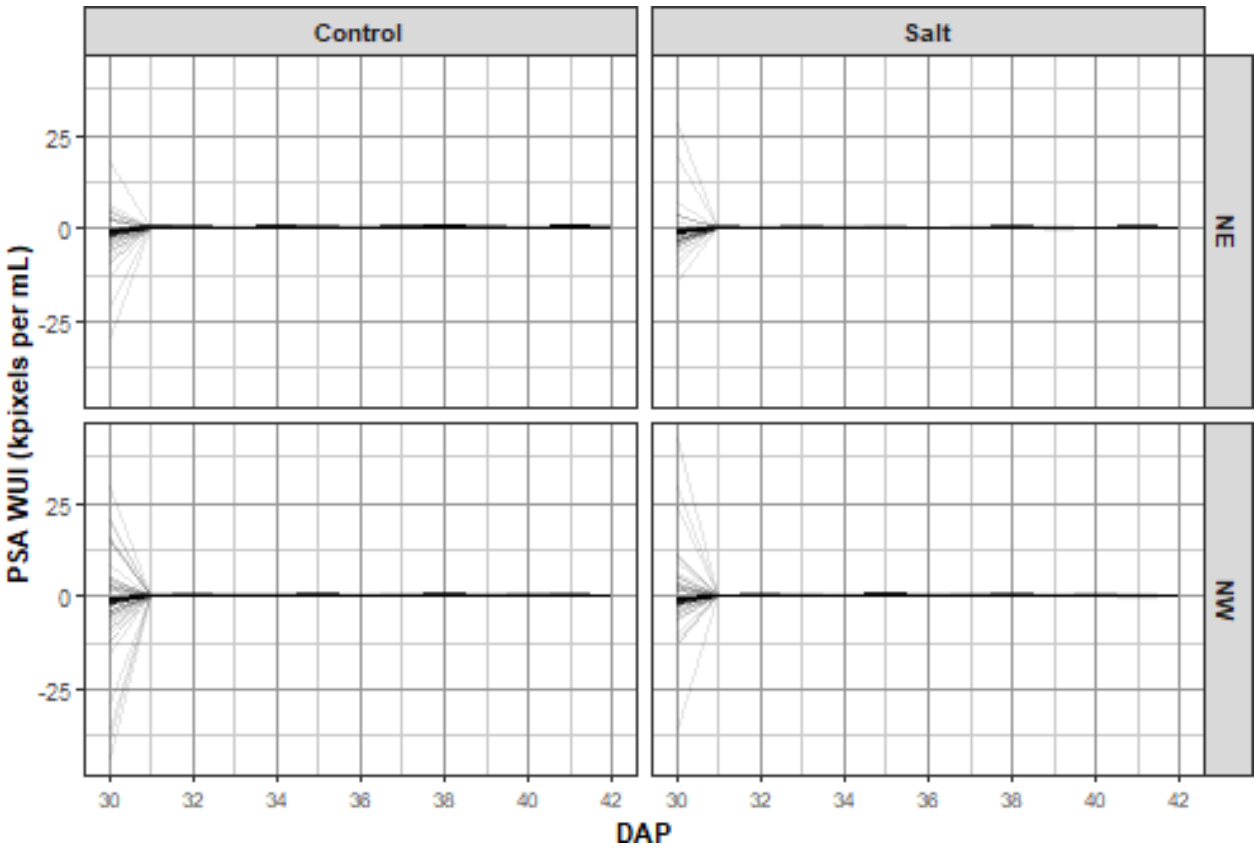

```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```




```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```

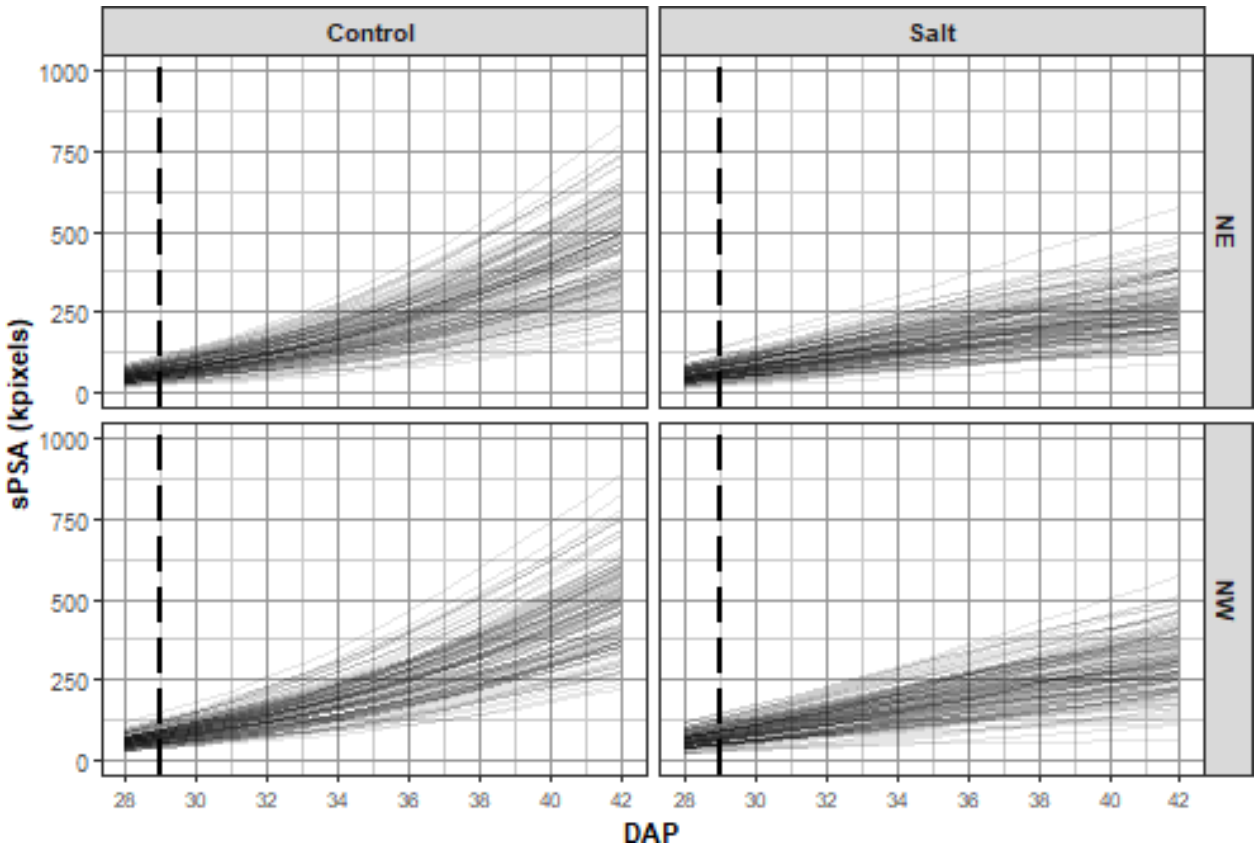



```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```

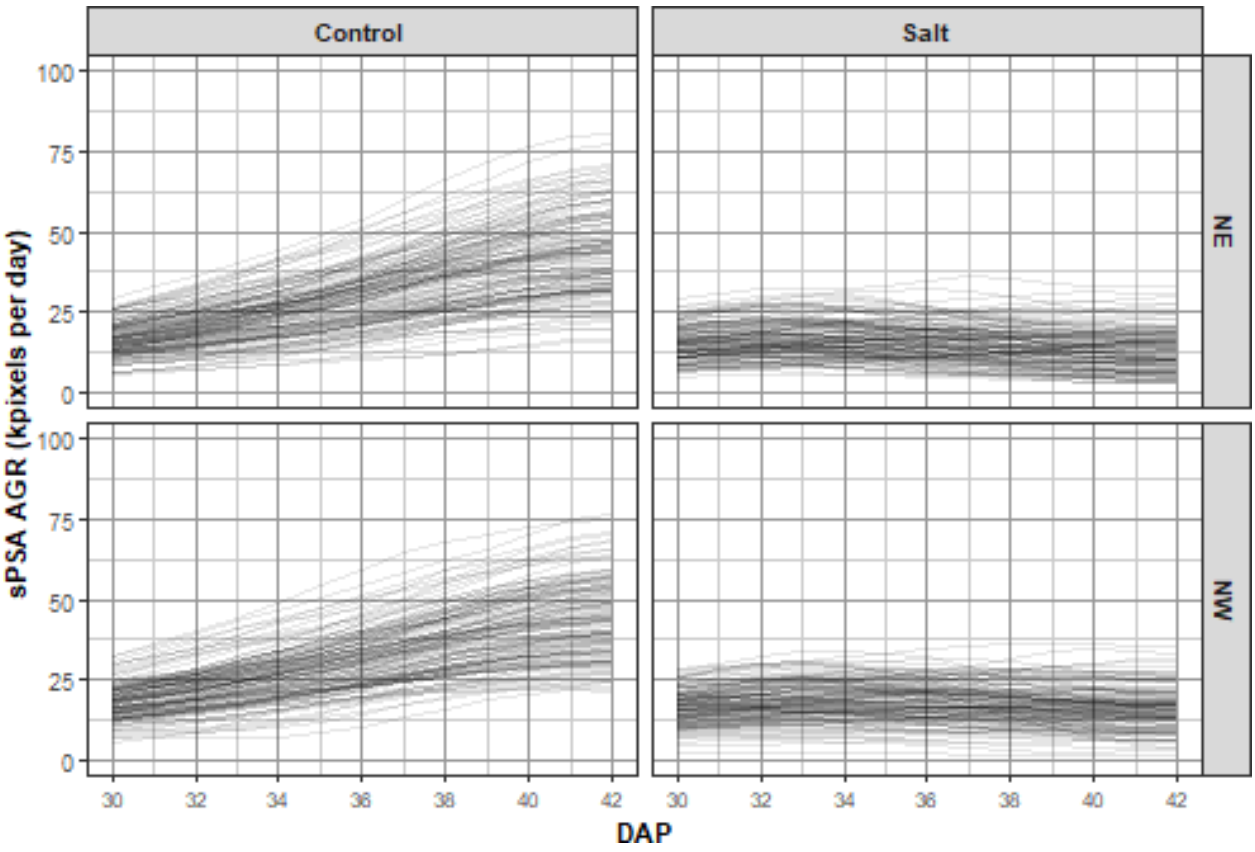


Plot smoothed profiles for all longitudinal responses

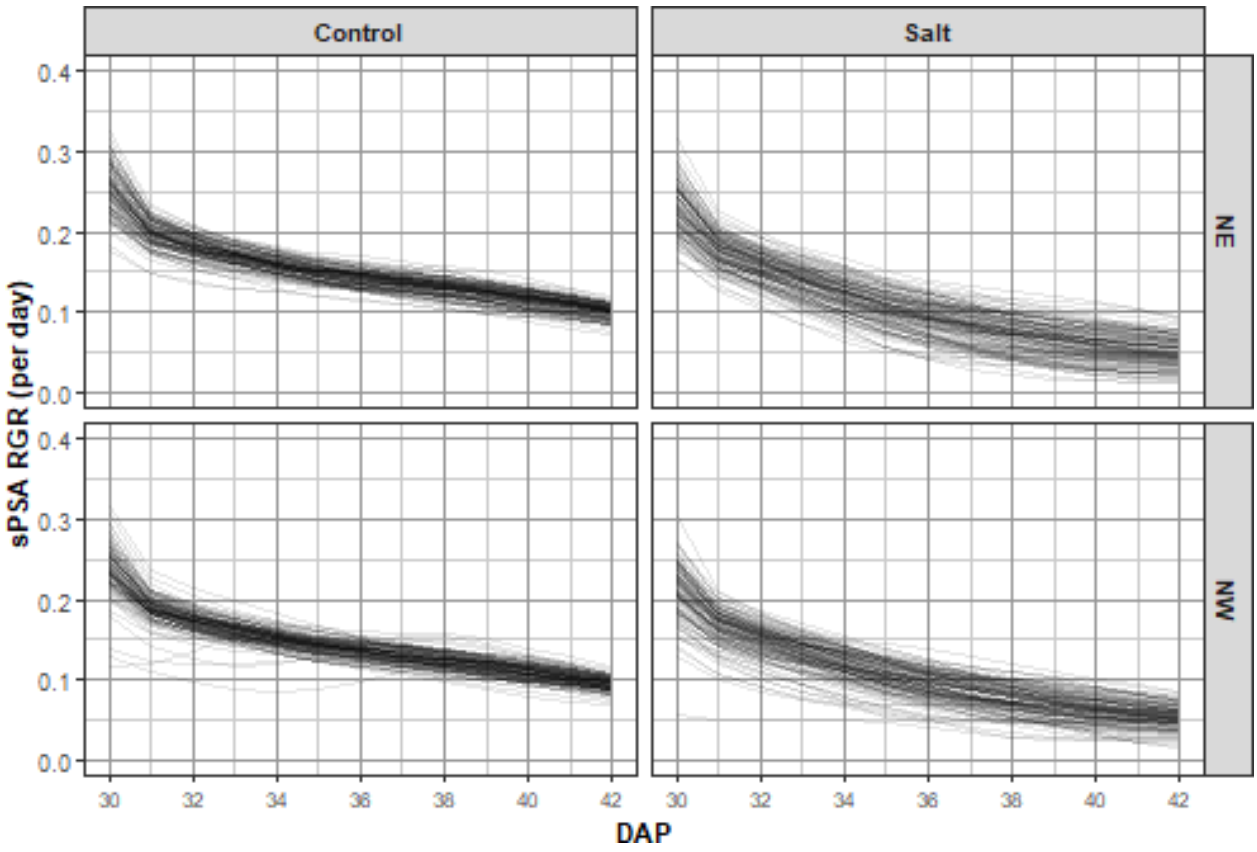
```
nresp.smooth <- length(responses.smooth.plot)
limits <- list(c(0,1000), c(0,100), c(0.0,0.40))
for (k in 1:nresp.smooth)
{
  plt <- plotProfiles(data = longi.dat, response = responses.smooth.plot[k],
                      y.title = titles.smooth[k], times = "DAP",
                      facet.x = "Treatment.1", facet.y = "Smarthouse",
                      breaks.spacing.x = 2,
                      printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1) +
               scale_y_continuous(limits=limits[[k]])
  print(plt)
}
```




```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```




```
## Warning: Removed 4 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```



Step 3: Choose the smoothing method and DF

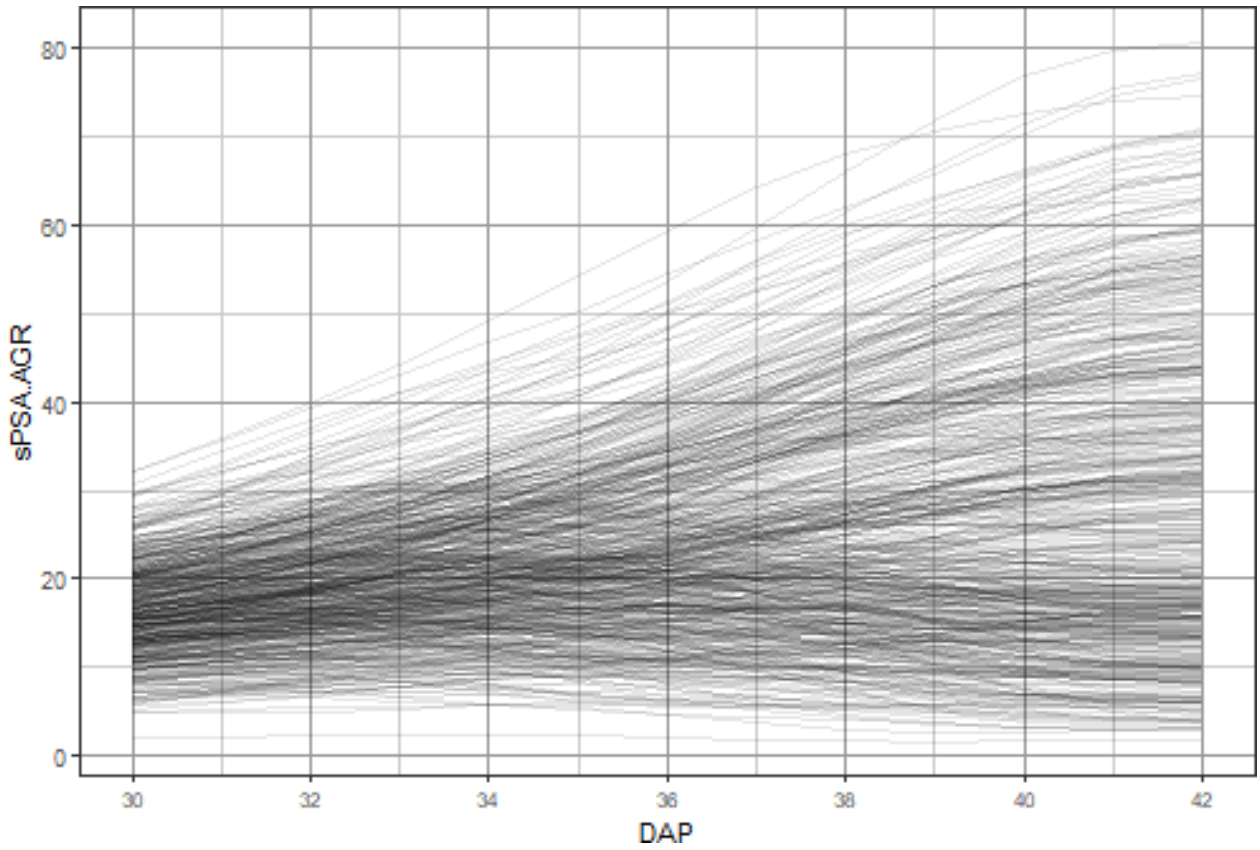
This step has been omitted.

Step 4: Identify potential outlers and clean the data

It has been decided that plants whose smoothed AGR are less than 2.5 after Day 40 are growing so slowly as to be considered anomalous. These plants are identified using `plotAnom`. Their values on Day 42 are printed. The plants are plotted without the anomalous plants followed by a plot of just the anomalous plants. The images of these anomlaous plants were examined and no particular problems were identified with them. They were retained in the data.

```
anom.ID <- vector(mode = "character", length = 0L)
response <- "sPSA.AGR"
cols.output <- c("Snapshot.ID.Tag", "Smarthouse", "Lane", "Position",
                 "Treatment.1", "Genotype.ID", "DAP")
anomalous <- plotAnom(longi.dat, response=response, lower=2.5, start.time=40,
                      times = "DAP", vertical.line=29, breaks.spacing.x = 2,
                      whichPrint=c("innerPlot"), y.title=response)
```

```
## Warning: Removed 1 row containing missing values or values outside the scale range
## (‘geom_vline()‘).
```

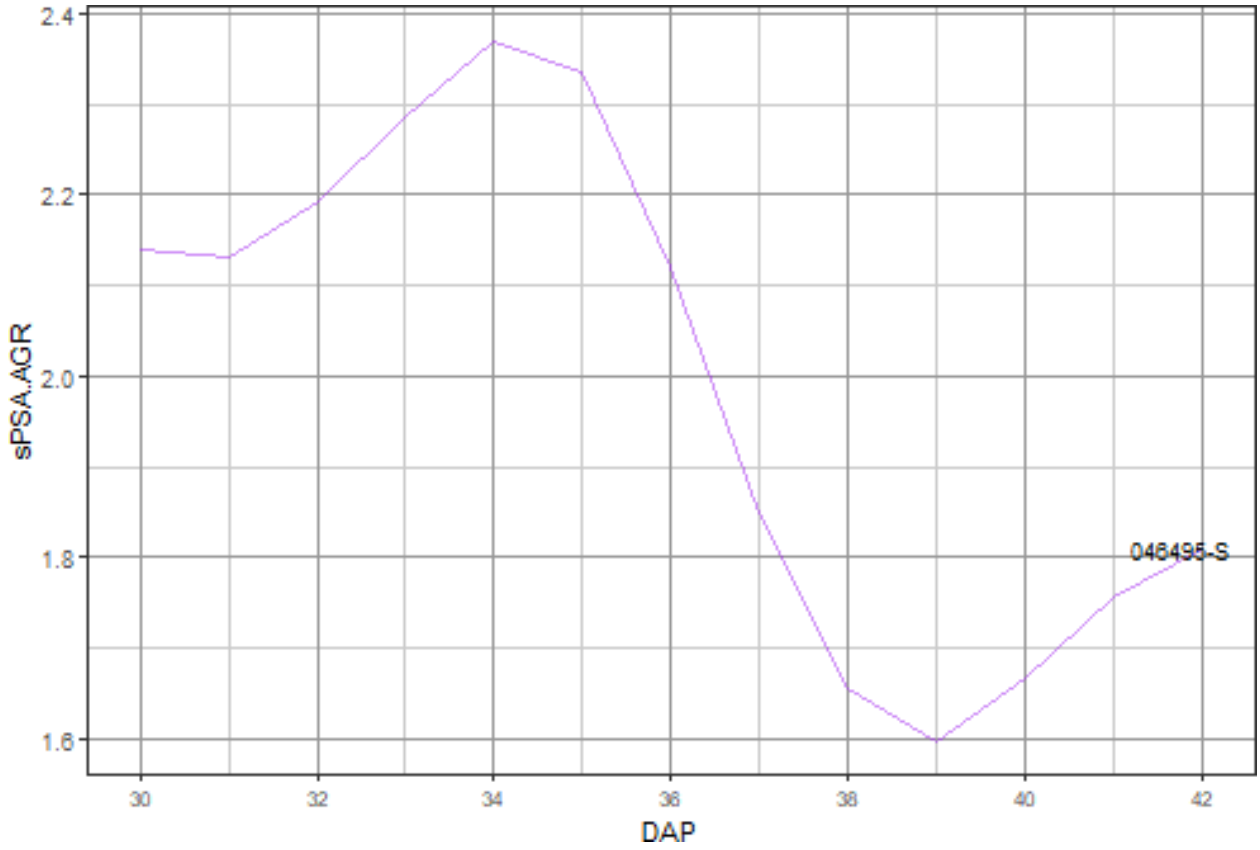



```
subs <- subset(anomalous$data, sPSA.AGR.anom & DAP==42)
if (nrow(subs) == 0)
{ cat("\n#### No anomalous data here\n\n")
} else
{
  subs <- subs[order(subs[["Smarthouse"]],subs[["Treatment.1"]], subs[[response]]),]
  print(subs[c(cols.output, response)])
  anom.ID <- unique(c(anom.ID, subs$Snapshot.ID.Tag))
  outerPlot <- anomalous$outerPlot  + geom_text(data=subs,
                                                aes_string(x = "DAP",
                                                           y = response,
                                                           label="Snapshot.ID.Tag"),
                                                size=3, hjust=0.7, vjust=0.5)
  print(outerPlot)
}
```

```
##      Snapshot.ID.Tag Smarthouse Lane Position Treatment.1 Genotype.ID DAP
## 6608         046495-S         NW   22       10        Salt      120952  42
##      sPSA.AGR
## 6608 1.809133
```

```
## Warning: `aes_string()` was deprecated in ggplot2 3.0.0.
## i Please use tidy evaluation idioms with `aes()`.
## i See also `vignette("ggplot2-in-packages")` for more information.
## Removed 1 row containing missing values or values outside the scale range
## (`geom_vline()`).
```

```
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```



Step 5: Extract per-cart traits

A range of single-value plant responses are formed in `Snapshot.ID.Tag` order.

#'### Set up intervals

```
DAP.endpts <- c(31,35,38,42)
DAP.starts <- c(31,35,31,38)
DAP.stops  <- c(35,38,38,42)
DAP.mids <- (DAP.starts + DAP.stops)/2
suffices <- paste(DAP.starts, DAP.stops, sep = "to")
```

Step 5(a): Set up a data frame with factors only

```
cart.dat <- longi.dat[longi.dat$DAP == DAP.endpts[1],
                      c("Smarthouse","Lane","Position","Snapshot.ID.Tag",
                        "cPosn","cMainPosn",
                        "Zone","cZone","SHZone","ZLane","ZMainunit", "Subunit",
                        "Genotype.ID","Treatment.1")]
cart.dat <- cart.dat[do.call(order, cart.dat), ]
```

Step 5(b): Get responses based on first and last date.

```
# Observation for first and last date
cart.dat <- cbind(cart.dat, getTimesSubset(data = longi.dat, responses = responses.image,
                                           times = "DAP", which.times = DAP.endpts[1],
                                           suffix = "first"))
cart.dat <- cbind(cart.dat, getTimesSubset(data = longi.dat, responses = responses.image,
                                           times = "DAP",
                                           which.times = DAP.endpts[length(DAP.endpts)],
                                           suffix = "last"))
cart.dat <- cbind(cart.dat, getTimesSubset(data = longi.dat, responses = "WUI.cum",
                                           times = "DAP",
                                           which.times = DAP.endpts[length(DAP.endpts)],
                                           suffix = "last"))
responses.smooth <- paste0("s", responses.image)
cart.dat <- cbind(cart.dat, getTimesSubset(data = longi.dat, responses = responses.smooth,
                                           times = "DAP", which.times = DAP.endpts[1],
                                           suffix = "first"))
cart.dat <- cbind(cart.dat, getTimesSubset(data = longi.dat, responses = responses.smooth,
                                           times = "DAP",
                                           which.times = DAP.endpts[length(DAP.endpts)],
                                           suffix = "last"))

# Growth rates over whole period.
(tottime <- DAP.endpts[length(DAP.endpts)] - DAP.endpts[1]) #= 11
```

```
## [1] 11
```

```
cart.dat <- within(cart.dat,
                   {
                     PSA.AGR.full <- (PSA.last - PSA.first)/tottime
                     PSA.RGR.full <- log(PSA.last / PSA.first)/tottime
                   })

# Calculate water index over whole period
  cart.dat <- merge(cart.dat,
                    byIndv4Intvl_WaterUse(data = longi.dat,
                                          water.use = "WU", response = "PSA",
                                          trait.types = c("WUI","WUR", "WU"),
                                          times = "DAP",
                                          start.time = DAP.endpts[1],
                                          end.time = DAP.endpts[length(DAP.endpts)]),
                    by = c("Snapshot.ID.Tag"))
```

Step 5(c): Add growth rates and water indices for intervals

```
# Growth rates for specific intervals from the smoothed data by differencing
for (r in responses.smooth)
{
  for (k in 1:length(suffices))
```

```
  {
    cart.dat <- merge(cart.dat,
                      byIndv4Intvl_GRsDiff(data = longi.dat, responses = r,
                                           times = "DAP",
                                           which.rates = c("AGR","RGR"),
                                           start.time = DAP.starts[k],
                                           end.time = DAP.stops[k],
                                           suffix.interval = suffices[k]),
                      by = "Snapshot.ID.Tag")
  }
}

# Water indices for specific intervals from the unsmoothed and smoothed data
for (k in 1:length(suffices))
{
    cart.dat <- merge(cart.dat,
                      byIndv4Intvl_WaterUse(data = longi.dat,
                                            water.use = "WU", responses = "PSA",
                                            times = "DAP",
                                            trait.types = c("WU","WUR","WUI"),
                                            start.time = DAP.starts[k],
                                            end.time = DAP.stops[k],
                                            suffix.interval = suffices[k]),
                      by = "Snapshot.ID.Tag")
}

cart.dat <- with(cart.dat, cart.dat[order(Snapshot.ID.Tag), ])
```

Form continuous and interval SIITs

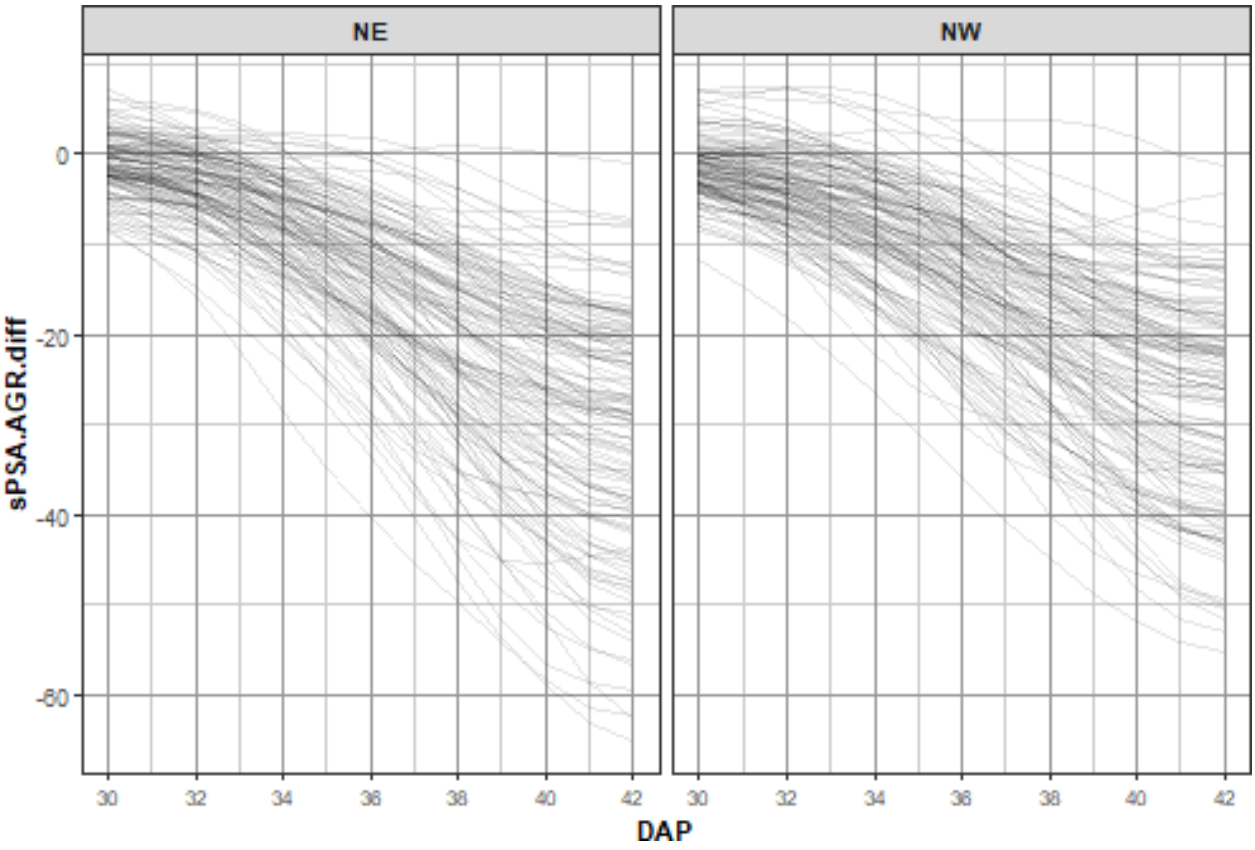
This experiment involved the extra step of calculating a measure of shoot ion-independent tolerance (SIIT) of pairs of plants, control and a salt-treated co-located plants.

Calculate continuous values

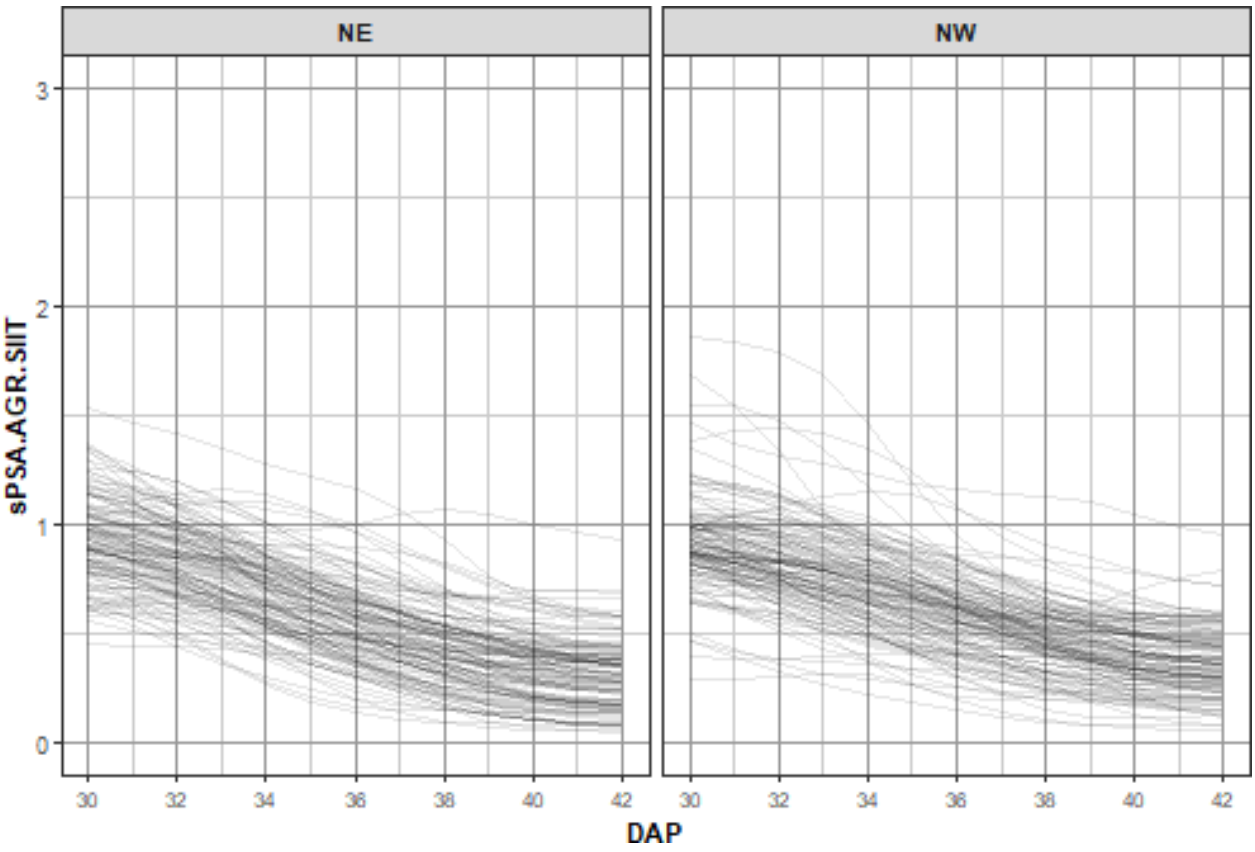
```
cols.retained <-  c("Snapshot.ID.Tag","Smarthouse","Lane","Position",
                    "DAP","Snapshot.Time.Stamp", "Hour", "xDAP",
                    "Zone","cZone","SHZone","ZLane","ZMainunit",
                    "cMainPosn", "Genotype.ID")
responses.GR <- c("sPSA.AGR","sPSA.AGR","sPSA.RGR")
suffices.results <- c("diff", "SIIT", "SIIT")
responses.SIIT <- unlist(Map(paste, responses.GR, suffices.results,sep="."))

longi.SIIT.dat <-
  twoLevelOpcreate(data = longi.dat, responses = responses.GR, suffices.treatment=c("C","S"),
                   operations = c("-", "/", "/"), suffices.results = suffices.results,
                   columns.retained = cols.retained,
                   by = c("Smarthouse","Zone","ZMainunit","DAP"))
longi.SIIT.dat <- with(longi.SIIT.dat,
                           longi.SIIT.dat[order(Smarthouse,Zone,ZMainunit,DAP),])
```

```r
# Plot SIIT profiles
k <- 2
nresp <- length(responses.SIIT)
limits <- with(longi.SIIT.dat, list(c(min(sPSA.AGR.diff, na.rm=TRUE),
                                      max(sPSA.AGR.diff, na.rm=TRUE)),
                                    c(0,3),
                                    c(0,1.5)))
#Plots
for (k in 1:nresp)
{
  plt <- plotProfiles(data = longi.SIIT.dat, times = "DAP",
                      response = responses.SIIT[k],
                      y.title=responses.SIIT[k],
                      facet.x="Smarthouse", facet.y=".",
                      breaks.spacing.x = 2, printPlot=FALSE, )
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", linewidth=1) +
               scale_y_continuous(limits=limits[[k]])
  print(plt)
}
```

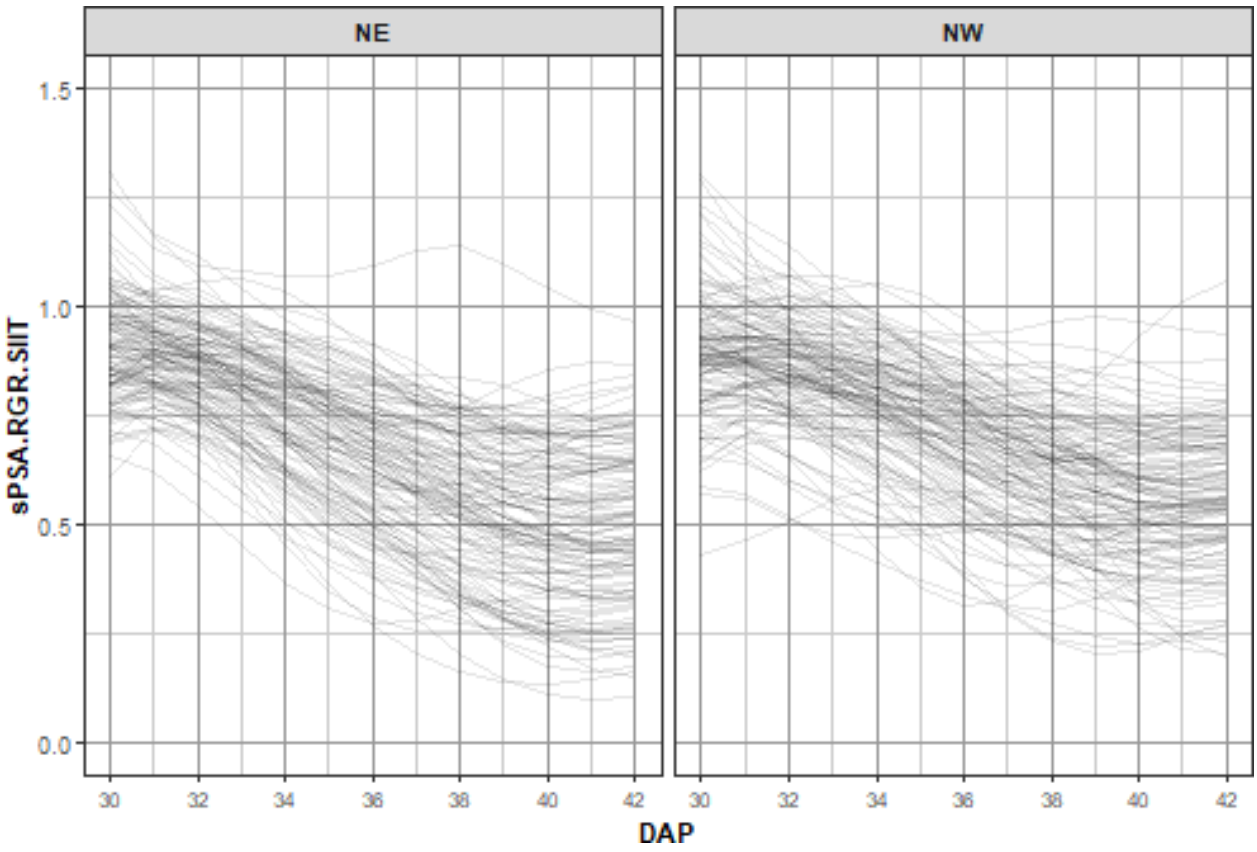
```
## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```




```
## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```




```
## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_vline()`).
```



Calculate interval SIITs and check for large values for SIIT for Days 31to35

```
response <- "sPSA.RGR.31to35"
SIIT <- paste(response, "SIIT", sep=".")
responses.SIITinterval <- as.vector(outer("sPSA.RGR", suffices, paste, sep="."))

cart.SIIT.dat <- twoLevelOpcreate(data = cart.dat, responses = responses.SIITinterval,
                                  suffices.treatment=c("C","S"),
                                  suffices.results="SIIT",
                                  columns.suffixed="Snapshot.ID.Tag")

tmp<-na.omit(cart.SIIT.dat)
print(summary(tmp[SIIT]))
```

```
##  sPSA.RGR.31to35.SIIT
##  Min.   :0.4240
##  1st Qu.:0.7240
##  Median :0.8033
##  Mean   :0.7940
##  3rd Qu.:0.8720
##  Max.   :1.0789
```

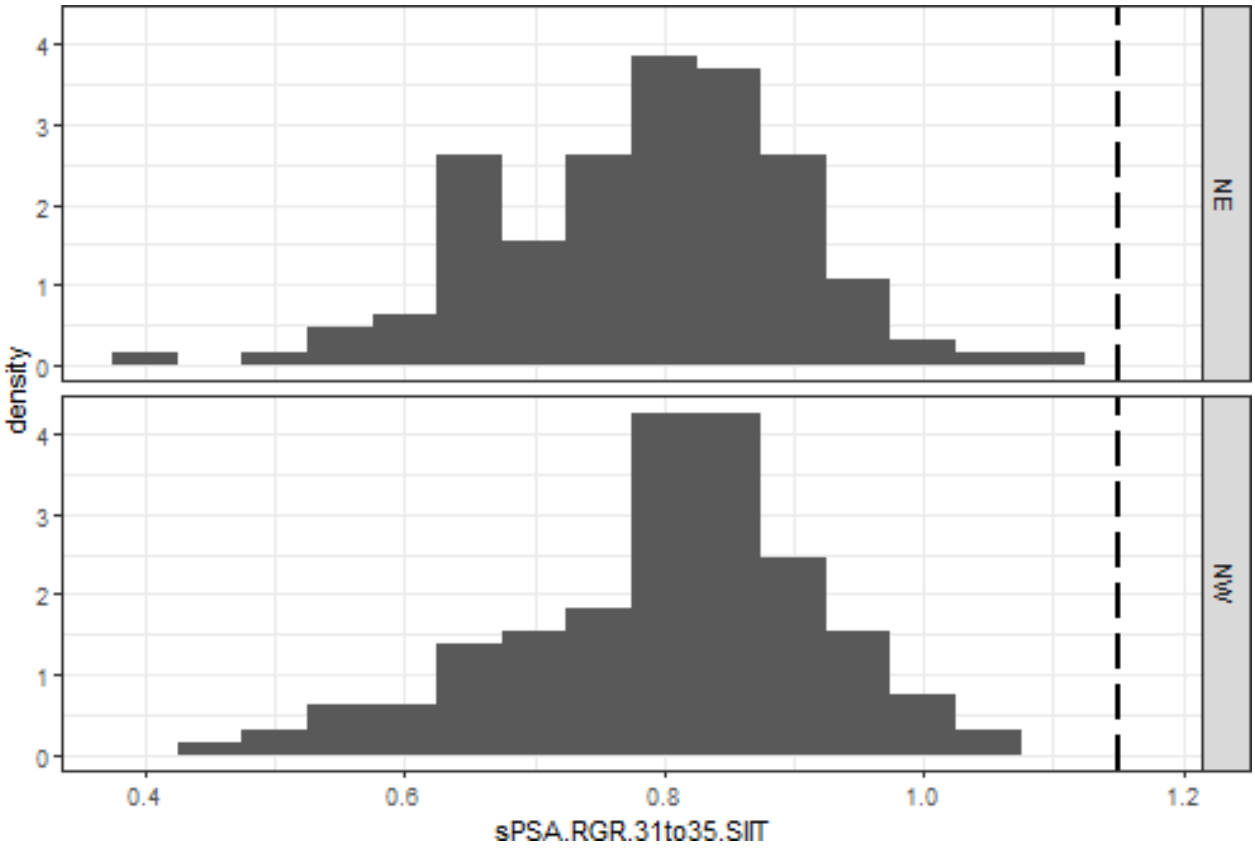
```
big.SIIT <- with(tmp, tmp[tmp[SIIT] > 1.15, c("Snapshot.ID.Tag.C","Genotype.ID",
                                              paste(response,"C",sep="."),
```

```
                                          paste(response,"S",sep="."), SIIT)])
if (nrow(big.SIIT) > 1)
  big.SIIT <- big.SIIT[order(big.SIIT[SIIT]),]
print(big.SIIT)
```

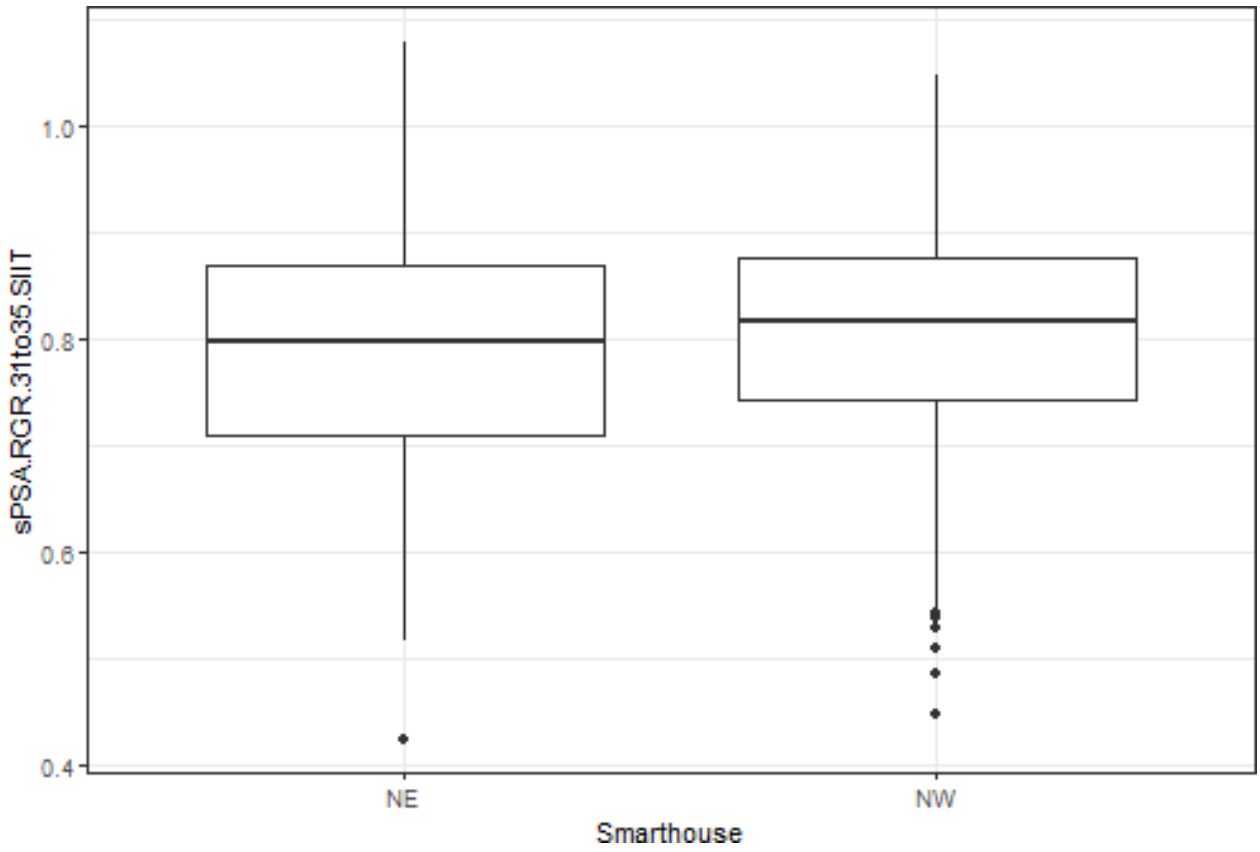
```
## [1] Snapshot.ID.Tag.C    Genotype.ID          sPSA.RGR.31to35.C
## [4] sPSA.RGR.31to35.S    sPSA.RGR.31to35.SIIT
## <0 rows> (or 0-length row.names)
```

```
plt <- ggplot(tmp, aes_string(SIIT)) +
           geom_histogram(aes(y = ..density..), binwidth=0.05) +
           geom_vline(xintercept=1.15, linetype="longdash", linewidth=1) +
           theme_bw() + facet_grid(Smarthouse ~.)
print(plt)
```

```
## Warning: The dot-dot notation (`..density..`) was deprecated in ggplot2 3.4.0.
## i Please use `after_stat(density)` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```




```
plt <- ggplot(tmp, aes_string(x="Smarthouse", y=SIIT)) +
           geom_boxplot() + theme_bw()
print(plt)
```




```
remove(tmp)
```

Save image

```
save.image("Rice.RData")
```

References

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Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J., & Thompson, R. (2023). *ASReml-R reference manual*, Version 4.2. http://asreml.org.

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