3-2015

# Development of a screening program for identification of the neoplastic pod gene (Np) in Syngenta snap peas

Emily Kniep
*University of Nebraska-Lincoln*

# Development of a screening program for identification of the neoplastic pod gene *(Np)* in Syngenta snap peas

**By**

**Emily Kniep**

**Master's Degree Project**

**March 2015**

**Summary**

Project Objective

The purpose of this project was to design a method for screening Syngenta snap pea varieties for the neoplastic podded gene (*np)*. Pea varieties will be characterized phenotypically using weevil extracts to produce a neoplasm response of the *np* gene. Screening for neoplasms will enable the breeding team to understand if the gene is present how it might or might not be affecting pod quality reductions.

Project Outcomes and Significance

The most important outcome was discovered during the proof of concept phase of protocol creation when it was established that the *np* gene is likely not the gene causing pod quality reductions. This is very significant because it eliminates a variable and allows for focus on other potential causes.

A second outcome was the fact that weevil extracts were not needed to verify if the *np* gene was present. Based on the initial work with the check varieties, the low light environment of the greenhouse was a very consistent inducer of the neoplasm response. Both a low light and a weevil extract protocol are included in this project. The weevil protocol is still a valid approach and was the initial direction for the project. The low light protocol is the more practical option for the breeding program and will be what is used this fall to verify that the *np* gene is in fact not the issue.

A third outcome was finding a direction for the next steps in the long term investigation into pod quality. A field in Washington was exposed to severe sand damage that had many of the same characteristics as the damage seen in California. Further testing will be conducted to explore sand damage and its impact on pod quality.

TABLE OF CONTENTS

**Introduction**

Snap peas (*Pisum sativum, macrocarpum*) have edible pods caused by reduced strings, thick pod wall membranes, and no parchment layer in the pod wall. Other types of peas, such as shell peas, contain strong strings and a parchment layer that makes consumption of the entire pod undesirable and requires removing the seeds from the pods for consumption. Three genes are responsible for the snap pea pod type (v, p, and n) that remove parchment and increase the membrane size. This pod type has been known for quite some time but hadn't been commercialized until the 1960's. Snap peas are quite similar to snow peas, differing in only in pod wall thickness. It is assumed that the modern snap pea varieties resulted from breeding projects to improve snow peas.

Since their introduction, snap peas have continued to gain popularity and acreage around the world is increasing to meet demands. Snap peas today are grown for both processed and fresh consumption. Much of the processing acreage in the US is in Idaho, Oregon, and Washington. Peas are harvested a single time by machine, processed through a plant where cleaning, sorting and blanching occur, and then packaged frozen. Packaging is typically smaller consumer freezer bags (alone or in vegetable mixes) or bulk for sale to other packaging companies. Fresh production in the US is primarily in California where peas are hand harvested, washed and sorted, and packaged in consumer fresh pack salad bags or bulk boxes, both destined for the grocery store. The processing market is mostly focused on yield and will tolerate lower pod quality because blanching and freezing the product can correct many deformities. Yield is very important to the fresh market as well but pod quality has a direct impact on yield. If the number of blemishes reaches an unacceptable level, the product is discarded or sold below value into the processing market. In snap peas yield can be defined as strictly number of pods on a plant or number of usable pods on a plant. Increasing the useable pods on a plant can involve multiple variables such as ease of pick, determinate maturity, and pod quality. Syngenta has been successful in selecting for peripheral yield traits except for pod quality which is not very well understood.

Diminishing pod quality is due to a multitude of variables including genetics, diseases, and cultural practices such as watering, harvesting, processing, shipping, etc. A potential cause for the reduction in pod quality is the neoplastic pod gene (*np*). It is a dominant gene associated with pea weevil (*Bruchus pisorum*) resistance in peas. The trait functions by causing a callus on the surface of the pod in response to pea weevil oviposition (Doss, et al., 1995). The response is designed to inhibit weevil entrance into the pod and ultimately the seed. This is an excellent resistance mechanism for the plant but from a pod quality standpoint, it is very destructive. Inheritance studies conducted by Nuttall and Lyall show that segregation between neoplastic and normal pods is consistent with single dominant gene control but they also found variation in the expression between plants within a population and between the same populations grown in the greenhouse versus the field. They concluded that light and humidity can impact the level of expression of the *Np* gene (1964). This expression can occur with or without weevil presence. Doss et al. describes two types of expression of the same gene, one caused by weevil oviposition and one caused by low light and humidity (1995).

When peas are grown in California in the winter or close to the coast, they can experience low light conditions and higher humidity which have the potential to cause a response of the *np* gene in those varieties dominant for the trait. Growers in the Salinas area where peas are heavily grown under these conditions frequently report pods with raised tissue blemishes, calling them "fog bumps." The blemishes described as "fog bumps" seem similar the blemishes caused by the *np* gene. Actual weevil pressure is not an in issue in California and they are easily controlled during seed production by chemical sprays at flowering and fumigation prior to seed storage, indicating breeding for the recessive phenotype is a potential solution for improving pod quality.

The purpose of this project was to design a screening program for the identification and characterization of the *np* gene in Syngenta snap pea varieties. Screening techniques are an important part of the overall strategy of a breeding program and are used to screen and select for or against many traits. At least two groups (Berdnikov, et al. and Doss et al.) have tested screening methods involving weevil extracts applied directly to pea pods for the purpose of phenotyping the *np* gene. These two studies were the basis for protocol design and were adapted with the intent to take small scale, proven studies and adapt them for use on a larger scale in a commercial breeding program. After the initial draft of the protocol, a series of observations were conducted to fine tune and confirm the procedure.

**Protocol Development**

Screening environment

The initial design was to perform the screen on pea plants grown in the greenhouse because it is easy to plant and manage, it can be done in the winter months when the crew is already working in the greenhouse, and it offers control of more variables. The first increase of the check varieties was performed in the greenhouse in January 2014. The two neoplastic checks spontaneously expressed shortly after pod formation and by prime pod most pods had excessive neoplasms. This spontaneous expression will make it difficult to use them as checks for confirming weevil solution efficacy.

Observation 1. Growing outdoors will prevent neoplasms from spontaneously expressing.

During the Doss study (1995), neoplasm formation was prevented by moving greenhouse plants outdoors at the onset of flowering. Based on the performance of the checks indoors during the increase, the inability to move pea plants outdoors in the Idaho winter months, and Doss's success with growing in an outside environment to prevent expression, the check varieties were grown outdoors and neoplasm formation was evaluated. Lacy Lady, dominant for neoplasms, was planted with the pea nursery 4/4/2014, on 30 inch beds at 75 seeds per plot. The plots were observed multiple times starting with flowering and continuing through dry down. There was a 100% absence of neoplasm expression and indicates growing in the field will prevent spontaneous neoplasms and is a good candidate for a screening environment.

Observation 2. Growing under excess lighting in the greenhouse will prevent spontaneous expression of neoplasms.

Growing in the field environment raises concerns about additional variables and makes the process more complicated for the crew. The peas were grown in the greenhouse with extra lighting to determine if the spontaneous expression due to low light could be prevented. 4 pots of Lacy Lady were planted on 10/15/2014 at 5 seeds per pot. Each pot was thinned to 4 plants after emergence. 2 pots were left on the benches under normal growing conditions and 2 pots were placed under intense lighting. For the intense light treatment, a Sun Blaze T5 High Output Fluorescent Lighting Fixture, designed specifically for growing plants, was purchased from a local indoor gardening store. A frame was built so that the plants were directly under the light bank. The lights installed were half growth stage and half bloom stage to mimic the overall greenhouse lighting set up which alternates high pressure sodium and metal halide. During the growing stages there were noticeable differences between the light treatments. Plants not under lights began flowering on 11/21 while plants under lights began flowering on 11/26. Neoplasm formation followed a similar pattern with no lights starting on 11/30 and lights starting 12/7. There was a noticeable reduction in neoplasm expression under the excess lighting, both with absence/presence on the pod and the severity of expression on those pods with neoplasms. While the check variety exhibited reduced neoplasm formation under the additional light treatment it still did express neoplasms, remaining unsuitable under these conditions as a check variety for the weevil solution.

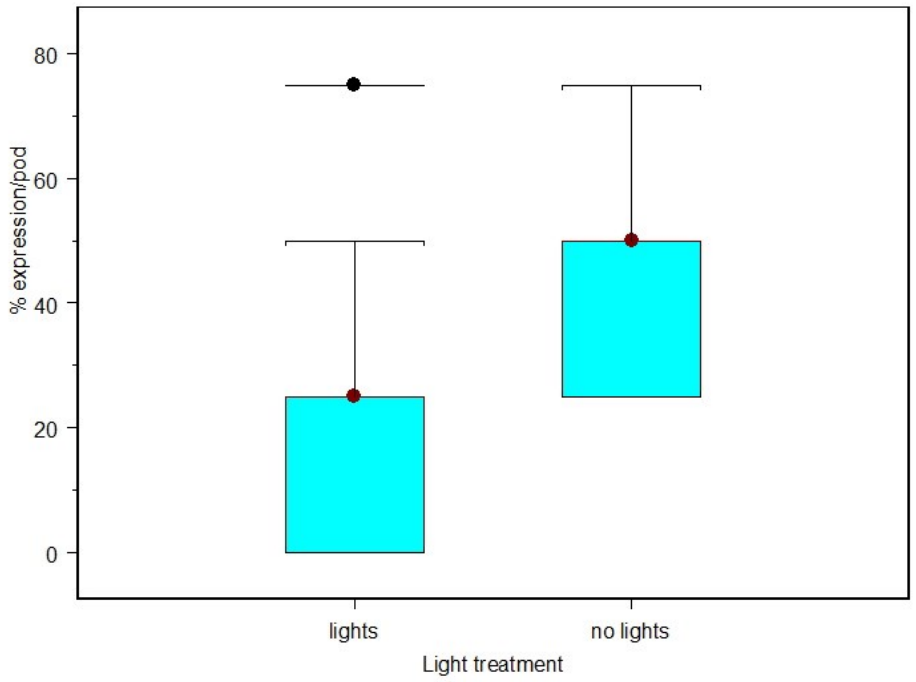



**Figure 1. Lacy Lady Neoplasm expression under different light treatments. Pods were counted and categorized by percent neoplasm coverage (0,25,50,75). The no light treatment had a mean of 43.24% neoplasms with no pods at 0%. Pods under extra lights had a mean of 23.98% and 29% of the pods without any neoplasm expression.**

Based on these 2 studies, the screening environment will be in the field in Idaho during typical pea production growing. Even with the potential for additional variables this is the best option given that the neoplasms do not spontaneously express with outside light conditions. The check varieties were tested outdoors twice, once in the regular pea breeding nursery and once in a fall planting, both with no neoplasm expression. As an additional quality check it will be important to monitor and record the behavior of the pods not treated with the solution during each planting to make sure there are no spontaneous neoplasm formations.

Weevil Solution

The weevil solution is a critical piece of the screening protocol. In the initial draft, weevils were to be collected in the spring from production pea fields, frozen, and used in the fall or winter for making the solution. Gender identification is visually very difficult so to compensate for this a large bulk of weevils is required to make the solution. This design will result in a mix of male and female weevils but will hopefully ensure that adequate females will be contained in the solution to produce a response. While pea weevils are present in Idaho, their economic impact is typically low enough in seed production that cultural management in the field during flowering is rarely needed, control being limited to fumigation of the seed after harvest. This also means that collecting weevils is difficult due to the negligible weevil population in the field. While using the sweep method for field collection of weevils during flowering in 2014, it was not possible to obtain the number of weevils required for the screen. This prompted investigating other options for weevil collection.

Observation 1. Sexually immature weevils collected from seed bags will cause neoplasm formation.

Weevils were collected from seed bags in August 2014. The papers indicated that sexually mature female weevils were best at causing an expression but the possibility of sexually immature weevils would be an easy alternative if they proved effective at also causing a response. Collecting from seed bags made it possible to acquire a sufficient number of weevils with relatively little effort. Also, identification of the weevils was no longer an issue making it easier for individuals with less experience to collect the correct insect.

Following the protocol, weevils were placed in the freezer after collection to kill them and store until use. Lacy Lady and Round Podded Sugar were planted in the field and allowed to reach flowering. Weevils were then bulked (200 weevils) and put into solution. The weevil solution was applied to the pea pods on 9/18/2014. Pods were observed on a daily basis through 10/2/14. On 9/24 it looked like neoplasms might be starting to appear on Lacy Lady but after another couple of days no neoplasms actually appeared. On 10/2/2014, well past when the neoplasms should have appeared the observation was ended without any neoplasms developing. This indicates that either the solution was made incorrectly, applied incorrectly, or sexually immature weevils might not be effective at causing an expression.

Observation 2. Weevils can be frozen and then removed from freezer and fed pea pollen to achieve sexual maturity.

While removing weevils from the freezer to make the first solution, it was discovered that the weevils had a high rate of cold tolerance. This presented the opportunity to attempt using recovered weevils from the freezer, reared to sexual maturity, and then used in the solution. After approximately 60 minutes out of the freezer, a portion of the weevils began moving limbs and trying to right themselves. The weevils were moved from the plastic freezer bag to a plastic cage with ventilation. After 24 hours more weevils were moving limbs but none had recovered enough that they were able to right themselves or move about the cage. At 48 hours after removing from the freezer the number of weevils recovering and the level of movement had plateaued. The weevils were still moving limbs and trying to right themselves but none had been successful. At 72 hours the number of weevils moving and the amount of movement was in a steep decline. The weevils were quickly dying and it was clear that they were not going to recover at all.

The appropriate method of weevil collection and solution creation has yet to be decided for the final protocol. The next option to be tested is will be to collect from seed bags in the fall, feed pea pollen to produce sexually mature weevils, and then freeze for future use. This is complicated because it requires having pea pollen ready when the weevils emerge from the seeds in the fall. It also requires weevils to be caged and maintained which increases resources and is outside of normal activities for the crew. It could be simplified by collecting a huge number of weevils one time and using them for multiple years after rearing to sexual maturity and freezing, but to do that the quality of the weevils based on storage time should be evaluated.

Rating Scale

Accurately measuring and recording the response in a way that that will aid in characterizing a variety as resistant or susceptible is critical for both short and long term use by the breeding program. The rating scale must be easy to understand and give clear direction about where to place each observation on the scale. The protocol was originally designed to evaluate at the sub plant level (individual pods) and capture severity of neoplasm expression. To date, the check varieties have exhibited a very clear presence/absence response and it was difficult to determine severity percentages. The most useful information for immediate use by the breeding program will be presence or absence per variety and severity is more of a nice to have. Based on this, the protocol is now designed to report on a variety level and each will be characterized as presence, absence, or segregating. Segregating will be determined on a plant level. Multiple plants per variety should be tested and using a higher number of pods per plant will provide confidence in the screen. Using this rating system will be easier to train the crew to evaluate and will be less subjective, making the results more consistent. Understanding the severity by variety might prove to be valuable and in the future it might be necessary to revisit and change the evaluation strategy to collect more information if differences are identified during the first screening trials.

**Future investigations**

While working through the validation of the protocol it became clear that first, the *np* gene is not likely the cause of pod quality reduction and second, if the *np* gene was the cause, there is no need

to use weevils to screen. In Syngenta, the breeding program is organized so that the majority of the varieties are grown in the greenhouse at one point or another. There are no Syngenta varieties in the current pipeline that exhibit neoplasm expression similar to Lacy Lady when grown in the greenhouse. This indicates the *np* gene is likely not the cause of the "fog bumps" observed by growers. Additionally, Lacy Lady consistently expresses neoplasms when grown in the greenhouse which suggests that growing in the greenhouse would be a sufficient screen for neoplasm expression.

**Figure 1. Lacy Lady, Nampa greenhouse 2/28/2014. Severity of neoplasms increases from left to right. This was a spontaneous expression due to low light in the greenhouse.**

To confirm that the *np* gene is not the cause of the defects observed, a formal screen of all of the commercial varieties should be performed. The described protocol using weevils could easily be abandoned and screening could be performed by simply using the low light environment to induce the neoplasm response, including the checks as verification that the greenhouse conditions are suitable to produce a response. If no neoplasm expression is observed under low light, fixed recessive for the *np* gene can be assumed. It is for this reason that two protocols have been provided. The weevil solution based protocol was included because that was the initial direction of the project but the low light protocol will serve as the final one that Syngenta will use to verify the *np* gene is not causing the pod quality issue. It is simple, straight forward, and will be an effective screen.

Because the *np* gene is likely not the cause of the pod quality issues observed in the production fields, the actual cause is still unknown. Further investigation is necessary to understand what factors are causing pod quality loss. One theory is mechanical damage due to wind and sand at critical times during pod formation. The pictures below were taken from production fields and are examples of what growers are observing. Each picture, taken in different locations, has similar characteristics for the pod deformities. They both have raised bumps and dark depressions. The second picture, taken in Washington is significant because there is a known event that can be linked to the damage. This damage has been coined "sand blasting" and was caused by strong winds where the pea field was downwind from a recently tilled field. The worst damage was closest to the tilled field and lessened

farther away from the loose soil. The wind storm occurred right after flowering at pin pod stage when the pods were small and delicate. In California, damage is usually in a pattern such as along one side, in a corner, etc. Similar cultural conditions such as tilled fields nearby are frequent due to the diversity in crops grown and growing cycles. At any point in time a field nearby can be in between crops or experiencing high traffic during planting or harvest. Peas grown in California could be experiencing low to medium levels of sand damage. Along with this damage there could be other pressures, such as pathogens, insects, humidity, light, and pollutants that could compound the damage and cause blemish variation.

**Figure 2. Picture was taken in a Sugar Lace field in California, 10/2/2013. Two types of deformities are present here. Fog bumps, indicated by a red circle, are raised bumps on the pod wall surface. The blue circle shows indents in the pod wall surface with possible bacterial growth (causing the dark coloration).**

**Figure 3. This picture is from a field In Pasco, Washington taken 6/11/14. The field was adjacent to an fallow field that had been tilled. During pod formation there was a strong wind event that blew loose dirt from the fallow field into the pea field.**

In order to survive adverse conditions, plants have developed physical barriers that limit damage and prevent secondary pathogen entry. This is innate or basal resistance and usually not pathogen specific, is quite complex, and has varied responses depending on the species, threat, and environmental conditions. These physical barriers are part of a plant's response to wounding that triggers a set of reactions designed to isolate the damage, prevent secondary infection, and repair affected tissue (Sanchez-Serrano, 2001). This occurs at the cell level instead which could also account for the multiple types of damage observed on individual pods.

Precheur, Greig, & Armbrust (1978) studied the effect of wind and sand on tomato plants and found that wind and wind plus sand can have a profound effect on yield and quality through morphological and physiological changes to the plant. The study found that sand exposure, even for short periods, caused injury to the epidermis and triggered the plants to develop a secondary epidermis and extensive wound callus at the injury site. Wounded cells also produced ethylene, which has been linked to cell swelling. Sand damage caused the tomato plants to compensate by increasing photosynthesis and respiration which in turn required an increase in the number of stomata. An increase in stomata numbers is important because if injured and not properly functioning they can be additional entry points for pathogens.

Snap peas pod characteristics such as a thick pod wall membrane, no fiber layer, and reduced suture strength are all alterations that impact plant productivity. Germination is reduced, pod size is smaller, pods are often curved, and pollen competition is reduced (McGee & Baggett, 1992). It is

plausible that these alterations could also affect how the pod epidermis cells react to wounding. Cuticle, waxes, stomatas, and cell wall characteristics all make up physical barriers that assist with passive defenses. Plants also have the ability to locally (on a cell level) recognize pathogens. This could account for the difference in responses to the sand damage (Brown et al., 1997). The neoplasm formation associated with weevil resistance is a type of hypersensitive reaction that is not unique to the *np* gene or to peas. It occurs in many different plant species and is a reaction to many threats such as viruses, fungi, bacteria, nematodes, and insects. The reaction is variable, sometimes leading to cell death but not always (Heath, 2000). Because peas already exhibit one form of hypersensitive reaction, it is a possibility that there are more instances of this in the species.

Wounding responses contributing to pod quality loss has not been well studied in any plant species and especially not in peas. Because so many physiological responses are occurring and it is probably polygenic, it will be extremely difficult to pinpoint exactly what is going on let alone breed for resistance. What will be important is gaining an understanding of what can be done culturally in the production environment to mitigate damage. With that in mind, the next step will be testing the theory of sandblast damage to assess the respond to damage and recovery from it. Varying the duration and intensity of sand exposure might provide some correlation between damage seen in Washington and California because it is assumed that one is acute damage while the other is more chronic. Adding common pea pathogen pressure as a variable will hopefully lead to an explanation for variation in deformities. Experimenting with maturity at the time of damage will be important to determine when peas are at their most vulnerable. If all else fails, experimenting with sand damage in snap peas would rule out yet another variable in the search for pod quality improvement.

References

Berdnikov, V.A., Trusov, Y.A., Bogdanova, V.S., Kosterin, O.E., Rozov, S.M., Nedel'kina, S.V., & Nikulina, Y.N. (1992). The neoplastic pod gene (*Np*) may be a factor for resistance to the pest *Bruchus pisorum* L. *Pisum Genetics,* 24, 37-39.

Brown, J. F. (John Frederick) & Ogle, H. J & Australasian Plant Pathology Society & Australasian Plant Pathology Society & Brown, J et al. (1997). *Plant pathogens and plant diseases*. Rockvale Publications for the Division of Botany, School of Rural Science and Natural Resources, University of New England, Armidale, N.S.W.

Doss, R.P., Proebstring, W.M., Potter, S.W., & Clement, S.L., (1995). Response of *Np* mutant of pea (*Pisum sativum* L.) to pea weevil (*Bruchus pisorum* L.) ovipostion and extracts. *Journal of Chemical Ecology*, 21(1), 97-106.

McGee, Rebecca. J., & Baggett, J. R. (1992). Inheritance of Stringless Pod in Pisum sativum L. *Journal of American Horticultural Society*, 628-632.

Nuttall, V.W. & Lyall, L.H. (1964). Inheritance of neoplastic pod in the pea. *The Journal of Heredity*, 184-186.

Precheur, Robert, Greig, J.K., & Armbrust, D.V. (1978). The Effects of Wind and Wind-Plus-Sand on Tomato Plants. *Journal of American Society for Horticulture,* 103(3), 351-355.

Sanchez-Serrano, Jose J. (2001). Plant Responses to Wounding. Encyclopedia of Life Sciences, John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1038/npg.els.0001321]

| Syngenta Seeds, Inc. | | SITE WORK INSTRUCTION | | |
|---|---|---|---|---|
| TITLE | | DOCUMENT NO. | REVISION | EFFECTIVE DATE |
| SNAP PEA NEOPLASM CHARACTERIZATION, WEEVIL | | | | |
| APPLICABLE TO | | PAGE: 1 | | |
| Legumes, NAFTA Snap Pea | | | | |
| DOCUMENT CREATED BY | REVIEWED BY | APPROVED BY | | |
| Emily Kniep | | | | |

## 1.0 Purpose

1.1 To provide instruction on conducting pea neoplasm characterization of fixed lines using a weevil solution.

## 2.0 Scope

2.1 NAFTA Syngenta Snap Peas. Trained personnel within the Global Legumes group.

## 3.0 References

3.1 N/A

## 4.0 Process-Specific Definitions

## 5.0 Materials and Equipment

5.1 Net for weevil collection

5.2 Cage for weevils (even a jar with a screen on the top will work)

5.3 Insect water gel (can buy from any pet store)

5.4 Mortar and pestle

5.5 Container with lid for weevil solution

5.6 Cotton balls

5.7 Check variety material (Lacy Lady, Round Podded Sugar)

5.8 Pea flowers/pollen

## 6.0 Safety

6.1 N/A

## 7.0 Procedure

7.1 PLANTING AND TRIAL SET UP

7.1.1 Plant in Idaho during standard planting windows to ensure healthy plants (Mid March – Mid April)

7.1.2 Options for screening trial: 1. Plant a specific trial: plant on 30 inch beds with a seed spacing of 2 inches. Plant at least 10 seeds per variety. 2. Select plants out of the Observation trail: Include check varieties with Observation trial and tag plants and pods within for screening.

7.1.3 Plant check varieties with each planting and plant enough to account for varying maturities (checks are Lacy Lady [neoplastic] and Round podded Sugar [non neoplastic])

7.2 WEEVIL SOLUTION

7.2.1 Collect weevils (Bruchus pisorum). See 8.1 in appendices for identification key. Two options for collecting weevils. Collection method 7.2.1.2 will be easier but will require more steps before solution can be created.

7.2.1.1 From a flowering Syngenta pea field in spring using sweep method. See 8.2 in appendices for sweep method. Make sure the field is flowering with beginning pod formation. Weevils collected in the spring from flowering pea fields can be considered sexually mature, required to produce a response.

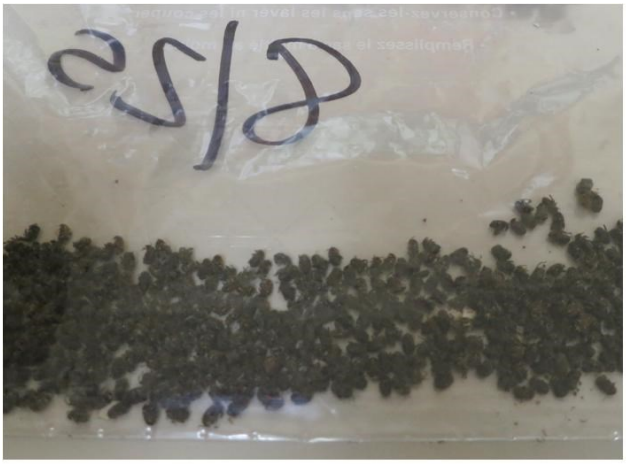
7.2.1.2 From harvested seed bags in August and July. After as short period post harvest, adult pea weevils will begin emerging from the seed. Open bags and collect emerging weevils. Weevils will not be sexually mature and will require pea pollen to reach maturity. Keep weevils in cages with ventilation and feed a diet of insect water gels and pea pollen (pick flowers each day and place in container) until eggs are noticed on the bottom of the cage, about 2 weeks. This will require a late planting in the field or greenhouse or freezing pollen during typical flowering times.

***Containing and feeding weevils has not yet been tested.
***Collecting and freezing pea pollen for feeding weevils has not been tested.

7.2.2 Store sexually mature weevils in freezer until ready to use.

7.2.3 Create solution at a rate of 0.1ml water/1 weevil. Make sure to use a bulk sample of weevils. i.e. 20ml water/200 weevils. You can also use a weight to make your solution (200 weevils = 2 grams)

7.2.4 Grind weevils in water using a mortar and pestle. Transfer to container with lid and shake vigorously to further blend.



Bag of weevils from freezer

Graduated cylinder for measuring water

1. Weevils in mortar before water

2. Grinding in process

3. Weevils ground, ready for application

7.3 APPLICATION OF SOLUTION

7.3.1 Select pods that are close to ½ size in maturity

7.3.1.1 About half the length of prime pod size (variety dependent)

7.3.1.2 Flat pods, seeds not visible

7.3.2 Randomly select 5 – 10 pods from each plant and attach label with date of application and solution number

7.3.3 Apply the solution using a cotton ball dipped in the solution. Make sure to wipe all parts of the pod. Allow to air dry.

7.3.4 Whenever possible only one solution should be used in an experiment and all solutions used should be applied to the check varieties as well as experimental entries. Always number and record solution on both experimental pods and check pods.


Tagged pod with date & solution number

Cotton ball dipped in solution

Applying solution to pod

7.4 RATING/EVALUATION

7.4.1 5 days after application evaluate pods for presence/absence of neoplasms

7.4.2 Expression categories:

7.4.2.1 SUSCEPTIBLE: Any presence of neoplasm expression (see picture below)

7.4.2.2 TOLERANT: no neoplasm expression

7.4.2.3 SEGREGATING: neoplasms segregating at the plant level (pods with and without on the same plant are okay – categorize as susceptible)

Examples of neoplasm severity.

7.4.3 Report results in the plot remarks column in SPIRIT (PSS:REMRK)

7.4.3.1 Concatenate date of evaluation, average and standard deviation of visual ratings, and reaction category.

7.4.3.1.1 Spirit Format: neoplasm screen;weevil 7-12-2014, resistant

## 8.0 Appendices

### 8.1 Pea Weevil Identification (http://www.grainscanada.gc.ca/storage-entrepose/pip-irp/pw-bp-eng.htm)

8.1.1 Classification

8.1.1.1 Primary pest; *Bruchus pisorum* (L.)
**Order:** Coleoptera
**Family:** Chrysomelidae

8.1.2 Description

8.1.2.1 Adults are 6 to 7 mm long, globular in shape with long legs

8.1.2.2 Elytra (wings) do not reach the end of the abdomen, leaving a small portion on the abdomen exposed

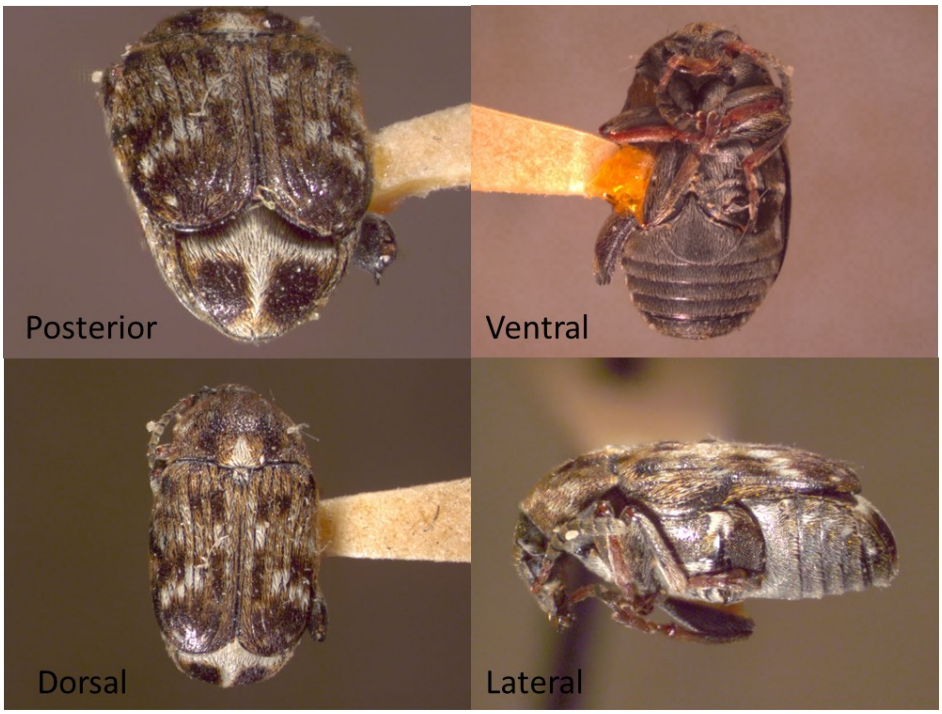
8.1.2.3 Last abdominal section is covered with black and white hairs and the inner ridge of the ventral margin of the hind femur has a single spine

8.1.3 Life history

8.1.3.1 Females lay eggs on outside of pod.

8.1.3.2 Larvae develop in growing seeds within pods.

8.1.3.3 After pupation within the seed, the adult chews an exit hole through the seed coat.




**8.2 Using a sweep net (www.gemplers.com)**

8.2.1 Hold the net with the hoop end nearest to the ground in front of you. The plane of the hoop should be perpendicular to you.

8.2.2 Swing the net from side to side in a full 180 degree arc. Sweep one stroke per step as you casually walk through the field or down the row.

8.2.3 Tilt the net opening so the lower edge of the rim is slightly ahead of the upper rim.

8.2.4 In short vegetation, swing the net as deeply as possible. In taller vegetation, sweep only deeply enough to keep the upper edge of the sweep net opening even with the top of the plants. In general, don't let the net go more than 10 inches below the top of the plants.

## 9.0 Record of Change

| Date | Revision | Description of Change | Change By |
|---|---|---|---|
| | | | |
| | | | |

| Syngenta Seeds, Inc. | | | SITE WORK INSTRUCTION | |
|---|---|---|---|---|
| TITLE | | DOCUMENT NO. | REVISION | EFFECTIVE DATE |
| SNAP PEA NEOPLASM CHARACTERIZATION, LOW LIGHT | | | | |
| APPLICABLE TO | | PAGE: 1 | | |
| Legumes, NAFTA Snap Pea | | | | |
| DOCUMENT CREATED BY | REVIEWED BY | APPROVED BY | | |
| | | | | |

## 1.0 Purpose

1.1 To provide instruction on conducting pea neoplasm formation characterization of fixed lines/varieties using a low light environment to induce a response.

## 2.0 Scope

2.1 NAFTA Syngenta Snap Peas. Trained personnel within the Global Legumes group.

## 3.0 References

3.1 N/A

## 4.0 Process-Specific Definitions

## 5.0 Materials and Equipment

5.1 Standard pea pots: 7x9in, 1.28 gal

5.2 Check variety material (Lacy Lady, Round Podded Sugar)

## 6.0 Safety

6.1 N/A

## 7.0 Procedure

7.1 PLANTING AND TRIAL SET UP

7.1.1 Grow in greenhouse following snap pea greenhouse protocol for lighting, potting soil, temperature, and watering/fertilizer schedule.

7.1.2 Pots – molded fiber 7x9in, 1.28 gal pots. Use cages or stakes as needed to maintain plant health.

7.1.3 For each variety, plant 1 pot with 4 plants in each pot (4 plants total). Overplant (plant 5 or 6 seeds and thin to 4) to ensure proper stand if needed.

7.1.4 Plant check varieties with each planting (Lacy Lady [neoplastic] and Round podded Sugar [non neoplastic])

7.2 RATING/EVALUATION

7.2.1 Monitor pods starting at flowering and continuing through prime pod

7.2.2 At prime pod evaluate for the presence of neoplasms

7.2.3 Expression categories:

7.2.3.1 SUSCEPTIBLE: Any presence of neoplasm expression

7.2.3.2 TOLERANT: no neoplasm expression

7.2.3.3 SEGREGATING: neoplasms segregating at the plant level (pods with and without on the same plant are okay – categorize as susceptible)

7.2.4 Report results in the plot remarks column in SPIRIT (PSS:REMRK)

7.2.4.1 Concatenate date of evaluation and reaction category.

7.2.4.1.1 Spirit Format: neoplasm screen; low light 7-12-2014, resistant

## 8.0 Forms

8.1 N/A

## 9.0 Record of Change

| Date | Revision | Description of Change | Change By |
|---|---|---|---|
| | | | |
| | | | |