BROOMRAPE EPIDEMIOLOGY AND INTEGRATED CONTROL

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Abstract

Sunflower broomrape caused by *Orobanche cumana* Wallr, as with other broomrape species, is very difficult to diagnose and control because this parasite has a long underground development stage. Despite several resistance genes being introduced into commercial varieties and hybrids, new virulent races continue to appear every time a new resistance gene is released. In developing and implementing an integrated control program we have encountered some difficulties, mainly coming from a lack of understanding of broomrape epidemiology and the prevalence of some myths well implanted in farmers and scientist mindsets. In this document some of these myths will be discussed and the most frequent patterns of field infection presented. A mathematical model corroborates that from the first infection in a field, to a very high infection stage, only three or four generations of sunflowers are needed to be grown in the same field. The infection pattern that can be observed in the fields each subsequent sunflower crop is the same irrespective if the crop rotation is two or nine years. The Syngenta sunflower integrated broomrape control program, now under the brand name SOLGUARD™, was presented at the last sunflower broomrape conference in Cordoba 2014. This included the use of three pillars: Genetic control; crop management and chemical control. The SOLGUARD™ program, through field race diagnosis, provides farmers with personalized plot by plot advice for the sustainable management of this parasitic weed. The reduction of primary infections, as well keeping the broomrape seed bank as low as possible, seems to be essential towards the eradication of this parasitic weed.

1. Introduction

Six broomrape species are regarded as either widespread or acute agricultural problems. ***O. crenata* Forsk.** Including *O. speciosa* D.C., ***O. cumana* Wallr.**; ***O. cernua* Loefl.; *P. ramosa* (L.) Pomel,** (Syn *O. ramosa* L., including *O. nana* Noe and *O. mutely* Schultz); ***P. aegyptiaca* (Pers.) Pomel**. (Syn. *O aegyptiaca* Pers.); and ***O. minor* Sm.** They are difficult to diagnose and control because these parasites have a long underground development stage, and by the time they emerge much of the damage has already been done. The long term impact of broomrape can be very serious: their seed may persist in soil for decades and their spread is facilitated by man, agricultural tools, planting seeds and animals, leading to an accelerated increase in the infested areas. In some countries there have been programs for the eradication or control of broomrape, such as the "Emergency plan for broomrape control in Israel" and "The branched broomrape eradication program in Australia" (Joel M., and Manor H. 2005; Warren Philip, 2005). Despite the failure of the Australian branched broomrape eradication program, this has provided a series of principles to manage the risks and to minimize the impact of broomrape infection applicable to sunflowers.As with many obligate parasites, sunflower broomrape produce numerous small seeds, which can survive in the soil for several years when non host crops are grown in the field, but only germinates when stimulated by the host root presence. The description of *Orobanchaceae* seeds and seedlings, germination stimulation and their germination ecophysiology have been reviewed recently, (See Joel, D.M., *et al*., 2013 chapters 8 to11) and this knowledge is essential to understand the epidemiology of broomrape. However, there have been few recorded stages on how a field is infected and how the broomrape seed bank builds in the soil.After the first observation in 1993, of a sunflower field with severe damage caused of Race F or sunflower broomrape, (Alonso L.C. *et al.,* 1996) we followed its expansion and distribution in Spain over the next 12 years (Fernandez-

Escobar J*., et al*., 2008). As the crop rotation in Southern Spain is very short, including often wheat after sunflowers and vice versa, these observations allowed us to understand better how new broomrape races are formed and what are the patterns of field infection. The Syngenta integrated sunflower broomrape control program was presented at the last sunflower broomrape conference (Alonso, L.C. 2014) and this included the use of three pillars: Genetic control; chemical control and crop management.

Genetic resistance genes are very difficult to find in most host species infected by any Orobanche specie, being the exception, the sunflower -*O. cumana* interactions as several resistant genes have been found in the wild species of *Helianthus* (Alonso. L.C., 1998).

Chemical control has only been possible in very few cases with the use of systemic herbicides that inhibit protein synthesis, such as the use in herbicide tolerant sunflowers of imidazolinones herbicides (Alonso *et al.*, 1998).

The use of trap crops or false hosts to induce suicide germination of broomrape seeds has been proposed, with different crops mentioned as trap crops of different *Orobanche* species (Goldwasser Y and J. Rodenburg 2013). In the case of *Orobanche cumana*, some trap crops have been recently published in Russia. (Antonova T. S., *et al.*, 2015).

In developing our program for a sustainable control of broomrape in sunflowers, we have found some difficulties mainly coming from lack of broomrape epidemiology understanding and the prevalence of some myths well implanted in farmers and scientists mindsets. In this presentation I will try to break some of these myths and bring an update of Syngenta's integrated broomrape program, now under our brand name SOLGUARD™.

2. Some common myths

2.1 Myth 1. A new sunflower broomrape race appears every 10 years and then expands!

This myth includes some other common thinking, such as the belief that ONLY one race is present in each field, and that broomrape races under the same letter (A, B, C…) are identical across the same country, or even across different countries. *Orobanche cumana* have been demonstrated to produce seeds by cross and self-pollination (Rodriguez-Ojeda, M.I. *et al.,* 2013), and there are indications of apomixis which have been observed (see Joel D.M., 2013 for review). These reproduction mechanisms, when combined with a probable enhanced mutation capacity, as has been observed in many obligate parasites, allow the broomrape to produce seeds of various genetic composition in each generation. Thus, new races evolve from previous races in infected fields, and under the same letter characterization there may be many different parasite mutants having in common their virulence on certain sunflower differential lines.

Some evidence point towards pre-existence of broomrape race F seeds prior to the use of sunflower hybrids resistant to race E. Broomrape seed samples collected in Spain at the end of the 1980s and identified as race E, had seeds of higher virulence. (Molinero-Ruiz *et al.,* 2008). Figure 1 (Alonso L.C. 2014) illustrates how preexisting broomrape mutations may be screened and selectively multiplied by the introduction of sunflower resistant hybrids, to become "new races". In subsequent generations, these new races will become dominant in the soil seed bank of the infected fields as they multiply, whereas the old races germinate and are unable to infect and as a subsequence they die.

2,2 Myth 2. Races with the same letter are identical across each country and from country to country.

The letter denomination of broomrape races indicate only that the broomrape isolates under the same letter are capable to infect certain "differential" sunflower lines. Two broomrape samples from different fields within a country or among countries may be classified as a race with the same letter and be different. In a recent study (Martin-Sanz *et al.*, 2016) it has been shown that broomrape isolates from different countries classified as race G, based on their virulence on race F resistant hybrids, can be further differentiated with the use of new sunflower differential lines, such as the inbred P-96. Whereas this line showed resistant reaction in a laboratory test using four broomrape isolates of race "G" from Spain, it was susceptible to broomrape race "G" from other Eastern Europe countries. Thus, the race "G" of these Spanish broomrape isolates were not introduced from other countries, but emerged locally from previous broomrape races in the fields. Furthermore, if P-96 is used as a new sunflower differential line resistant to race G, the Spanish broomrape isolates used in this study could be classified as race G and the others from East Europe as race H. (Table 1).

2,3 Myth 3. The most common way broomrape seed disperses is by wind

Broomrape seed can disperse by wind as they are very small. The myth is to believe that wind can disperse broomrape seeds uniformly across an entire infected field and to neighboring fields, as with a windborne disease, due to its small size. Flying a long distance from the last successful infection does not seem to be a good strategy for an obligate parasite. *Artemisia spp*., the wild natural host of *Orobanche cumana,* often appears in patches in the field. Most ripe broomrape seeds fall to the ground landing within half a meter of the parent plant (Warren P., 2005). Broomrape seeds have developed some traits to avoid flying-long distances. They are quite electrostatic in order to cling to soil particles as soon as they touch them. Also, they have a seed surface quite wrinkled-alveolated instead of flint resembling a golf ball. The dimpled broomrape seed surface may help it to fall more straight to the soil, pulled by gravity, than if the seed surface were flint.

3. The pattern of broomrape field infection and the number of years required for it.

Observations made in different farmers' fields during the last 30 years in Spain, and in sunflower trials planted in naturally infected broomrape fields, have helped to set a model for the broomrape soil seed bank built. *i.e*., the way a sunflower field evolves from the first infections of a new race to a field in which the broomrape seed bank is so large that it is not possible to grow sunflower without serious yield losses. This pattern has been possible to deduct because in Spain farmers often follow a very short rotation such as wheat/sunflowers or even repeating sunflowers after sunflowers in some years.

Table 2 & Figure 2 includes the 4 infection levels frequently observed in a field after the first infections, or a new race evolving inside an infected field planted with a new resistant sunflower hybrid. The main reasons for this pattern of development are the fact that most broomrape seeds fall and stay in the soil close to the infected plant and disperse with the help of conventional tillage. The progress of broomrape infection under non tillage is clearly much slower than under conventional tillage.

Level 1. Infection only occurs in few individual plants per hectare, not in groups. These individual plants are often distributed at random in the field. This is often observed in a heavily infected field when planting a new broomrape resistant hybrid. Often this level of infection passes unnoticed by farmers and even technical experts and as there is no damage in the crop everyone is happy with the control provided by the new resistance. However, broomrape plays an evil strategy. It makes everyone believe it does not exist until it is too late, as these individual infected plants may be screening and multiplying a new broomrape race.

Level 2. Can be observed**,** next time sunflower is planted in a field that had level 1 infection. Due to traditional tillage, small groups of plants infected are often consecutive in the same row when plowing and planting was done in the same direction. When plowing and planting are made in cross directions, the consecutive infected plants are often in the direction of the plowing. Also in these fields, one can see many individual plants infected with a random distribution. This infection level causes insignificant damage to the crop and farmers often do not see it.

Level 3. Broomrape infection appears in relatively small patches. More broomrape shoots/plant are observed in the center of these patches. The interpretation is that these patches are formed from the cumulative broomrape seed from the previous sunflower crop, with consecutive infected plants in the Level 2 infected field. As in the level 2 infected field, there are also individually infected sunflower plants, also groups of consecutive infected plants, as well as single infected plants observed across the plot in a level 3 infected field. Field damage and farmers awareness depends on the number of patches and their size, but often some damage is observed. However, the sunflower yield may still be good

Level 4. Broomrape infection becomes generalized with large patches of sunflower plants with multiple broomrape shoots. Plants in the center of these patches have shorter height and delayed flowering as they have more broomrape shoots/plant than in the border of the patches. These large patches often overlap giving the impression of generalized field infection. Crop damage is evident, and farmers often call only when they see this high level of infection. Growing sunflower crop in these level 4 infected fields is not possible unless using broomrape chemical control with Clearfield hybrids plus IMI herbicides, or with a new genetic resistant hybrid.

The spread pattern in patches demonstrates that the broomrape seeds don't travel much once they fall to the soil

4. Mathematical model to explain the 4 levels of infection

In order to see the impact of the different things that can influence the broomrape seed bank build up in the soil, the following formula has been used starting from a single infection per ha.

$\mathbf{N(m)} = \mathbf{A(m\text{-}}1) \times \mathbf{Y}^{n} \times \mathbf{Z}$

Where
Nm = Number of broomrape shoots per ha in the "**m**" sunflower crop after 1rst infection
Am = Nº seed produced.
Am = **X** x **Nm**, where the **X** variable = number of seed produced by a broomrape plant. For modeling purposed we have considered 1 plant to produce 30.000 seeds. Hence
Am= 30.000 x **Nm**

Y= Survival seed rate/year = This variable is considered 90% of previous year soil seed bank for modeling purposes i.e.,
Survival seed bank in year **n** = **Am** x $\mathbf{Y}^{n}$
Example: if only N = 1, the number of broomrape seeds in the field would decline until the next sunflower crop is planted, being **n** the number of years after the sunflower crops

year 1 = **A x Y** = 30.000 x 90%;
year 2 = **A** x $\mathbf{Y}^{2}$ = 30.000 x 81%
year 3 = **A** x $\mathbf{Y}^{3}$ = 30.000 x 73%

Z = Successful infection rate variable. For modeling purposes 2% has been used. i.e., out of the total broomrape soil seed bank only 2% infect the crop.

In **table 3,** the results of applying these formulas in 2 and 9 year rotation periods, In both cases, there is a sudden growth of the broomrape seed bank in the soil in the 4^{th} year to numbers well above the potential capacity of the sunflower crop to tolerate so many infections (see **figure 3**). We can conclude that

Period to reach Level 4 = (3 or 4) x Nº of years between sunflower crops

These results are very coincident with our observation in actual fields, and are similar to the results obtained by other mathematical models showing a fivefold increase of the seed bank every four seasons (Goldwasser Y. and J. Rodenburg 2013)

5. SOLGUARD™: The Syngenta´s sustainable broomrape management program

The SOLGUARD™ program has been launched by Syngenta in several CIS and Eastern Europe countries, with three main objectives. Long term reduction of damage on infected farms; prevent the spread of broomrape seeds and a gradual de-infestation of fields. Based on three pillars, Genetic resistance, chemical control and good agronomic practices, the benefits of this integrated approach include: Prevent broomrape introduction into non infected fields, avoid dispersion and race evolution, reducing the seed stock in the soil of infected fields, contributing to limiting the new virulent races of broomrape and integrate weed management and broomrape control. The main tool for the implementation of SOLGUARD™ program is based on Syngenta's exclusive App that includes tailored recommendations to farmers including personalized advice based on field by field diagnosis. It also allows data collection on farmers' broomrape infestation levels and race, creating a country map on broomrape and developing our knowledge of broomrape by area to deliver better recommendations year on year.

SOLGUARD™ broomrape race diagnosis per farm plot includes: Before planting diagnosis; field survey diagnosis during the crop growth and laboratory test after the crop is harvested.

The before planting diagnosis is based on a heuristic procedure through the use of a questionnaire, Syngenta's broomrape country/province risk map and other elements and allows to get the most probable race present in a given field. The App also produces a personalized recommendation plot by plot that is sent to the farmer. This heuristic diagnosis, has been proven to be correct so far in the great majority (>95%) of the fields we have tested.

The Apps also has the possibility to make a field diagnosis during or after flowering of the sunflower crop with the participation of Syngenta's technical experts. This can be done either to confirm or correct the results of the heuristic diagnosis or to evaluate a field that has never been diagnosed. The App utilizes both the field expert observations through a questionnaire plus the heuristic procedure used in the heuristic diagnosis. The level of accuracy of this field diagnosis is near to 100%. The App is also designed to help the field experts to assess the nature of infection in those cases where some infection is found due too many causes other than the buildup of new broomrape races. i.e., off types, escapees from herbicide treatment, etc.

Whenever required, Syngenta's field experts can collect broomrape seeds in any farm plot and send it to our laboratories for precise race diagnosis through the broomrape test using differential sunflower lines.

The use of the three pillars, genetic resistance, chemical control and good practices, differentiates Syngenta's SOLGUARD® program.

5.1 Genetic resistance.

Many growers, under the advice of seed companies, often decide to plant the variety or hybrid with resistance to the largest number of races. Due to the lack of accepted set of differentials, this has created an inflation of new letters, driven by marketing of seed companies, to define the latest races and the resistance to them. The results we can observe is a growing number of broomrape races or letters plus a large confusion on how to control them. Using the latest resistance gene may not be required in many cases, and using them alone in fields very heavily infected (level 4) only facilitates the appearance of new races. Furthermore, hybrids with the highest broomrape resistance may not be the best one in terms of adaptation and yield for a given region.

SOLGUARS™ recommends using the best adapted sunflower hybrid with the highest yield potential in the region with the capability to control the suspected dominant race in the farmer's field. Using the right resistance along with other control methods, may be enough to reduce the broomrape seed bank in the soil and keep the latest resistance to be used when really needed.

5.2 Chemical control

Clearfield® herbicides have been proven to control broomrape, while also providing good weed control. In many cases, when Clearfield® herbicide is applied it is difficult to determine the broomrape race present in the field. Also, there are examples where broomrape control is only done with Clearfield® herbicides. SOLGUARD™ app can provide heuristic broomrape race diagnosis, even when the herbicide has been applied. The service also allows monitoring of the potential buildup of weeds and broomrape resistance to Clearfield® herbicides.

5.3 Good practices

Often these are mentioned as educational tools but fail to be incorporated into the integrated control. Syngenta's SOLGUARD™ program incorporates some of these good practices in the recommendations sent to famers, along with the above mentioned genetic resistance and chemical control. There are two main situations where these good practices are incorporated in the recommendations. (Figure 4)

1. Preventing primary infections either in non-infected regions of the country or in certain plots within large farms.
2. Keep the primary infection for as long as possible at Level 1 or even move it to level 0

6. Is it possible to eradicate broomrape once a field is infected?

The United States Department of Agriculture (USDA) has been successful in the eradication of a parasitic weed similar to broomrape, *Striga* in corn. The first corn field infected by this parasitic weed was observed in US in 1956. The eradication program spent 250 mio US $ during 45 years (1956-2001). By 1999, the US corn area affected by *Striga* was 2800 Ha and after 2012 the parasitic plant is considered eradicated. (Eplee R.E. 1981; Parket C, 2012) This example demonstrates, eradicating parasitic weeds is possible.

But so far the attempts to eradicate broomrape have had limited success. There have been programs for the eradication or control broomrape such as the "Emergency plan for broomrape control in Israel" and "The branched broomrape eradication program in Australia" (Joel M., and Manor H. 2005; Warren Philip, 2005). The national branched broomrape program in Australia started soon after a small area was discovered in 1992 infected by branched broomrape in South Australia. The eradication program was State funded using methyl bromide, as soil fumigant, to destroy broomrape soil seed bank in the infected area. When it was found that the area affected was more widely distributed than first thought the National program was established in 1999 and the eradication program was adopted in 2001/02. Eradication remained the optimum strategy objective for years. But in 2011 the Australian Branched Broomrape National Management Group agreed that it was no longer technically feasible to eradicate the parasitic weed and the eradication program was wound up by end 2011.

I can bring an example of broomrape eradication in a farm in Spain. In 2002, one of the sunflower hybrids seed producing farms observed a plot with level 4 infection that was probably first time contaminated in 1995 during a drought as the farm is normally under irrigation but had limited water resources in that year. Under our advice, the farm adopted a very rigorous integrated approach. Sunflower seed production crops were only planted every five years and always using Clearfield® hybrids where application of the Clearfield® herbicide was mandatory. Furthermore, in the 5 year rotation at least 2 trap crops were included. i.e., Corn and cotton. In 2016, after 3 cycles using the above mentioned approach, we planted a hybrid seed production of a conventional hybrid. The plot was inspected several times without observing a single broomrape shoot. The farm may be re-infected in the future, as broomrape is present in the area, but keeping the two trap crops in the rotation and using Clearfield® resistant hybrids, at least once every two sunflower crops, should be enough to reduce the broomrape soil seed bank or keep the plots free of broomrape in non-infected areas.

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TABLES AND FIGURES

Figure 1. Preexisting broomrape mutations may be screened and selectively multiplied to become "new races" by the introduction of resistanthybrids

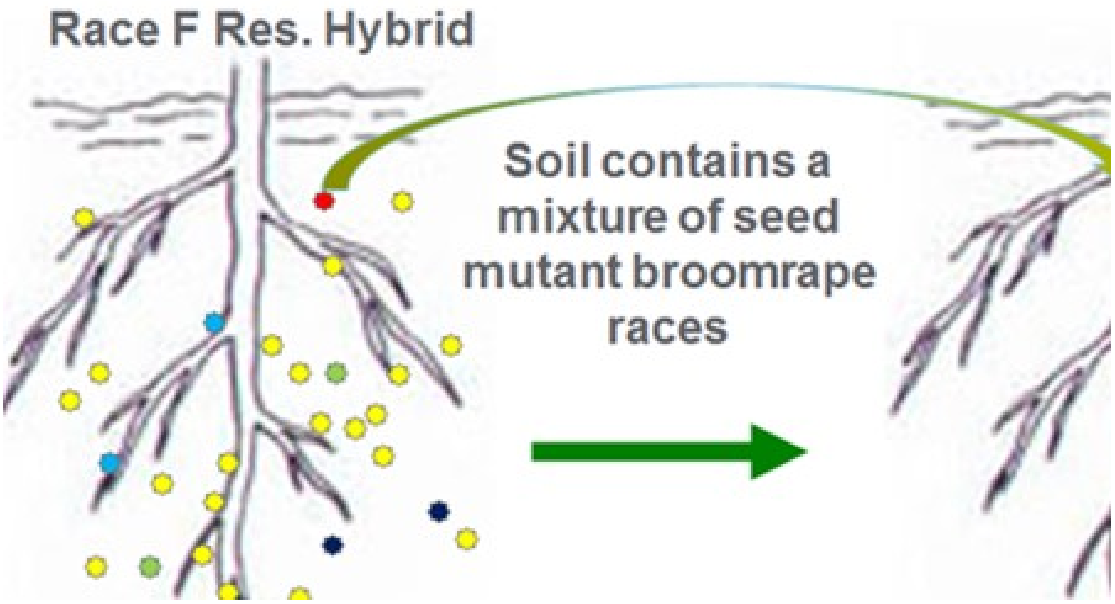
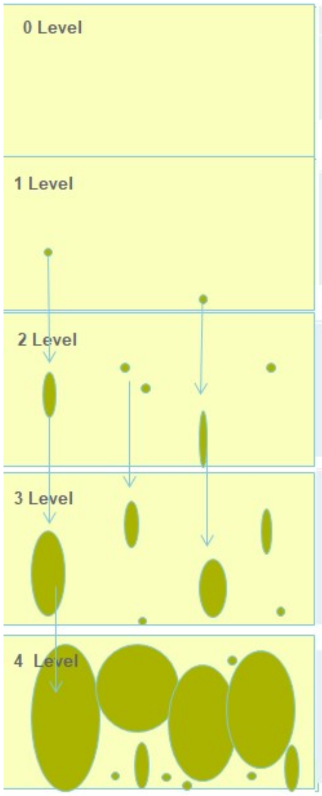


Table 1. Recent *O. Cumana r*ace G from Spain can be differentiated from race G of several East Europe countries (adapted from Martin-Sanz *et al.,* 2016*)*

	Broomrape race	
Sunflower differential	G Spain	*G East Europe*
Resistant to F	Susceptible	*Susceptible*
P-96	Resistant	*Susceptible*

Table 2 & Figure 2. Sunflower's broomrape epidemiology and field infection levels in subsequent sunflower crops



Infection level	Description	Crop Damage
0	There is no broomrape in the field	No crop damage
1	Infection only in few individual plants per Ha, not in groups, randomly distributed.	No crop damage
2	Small groups of plants infected in the same row. Isolated infected individual plants also present	Insignificant crop damage
3	Infections in small patches. More broomrape shoots/plant in the center of patch. Field damage may depend on the number and size of patches	Some crop damage
4	Infection becomes generalized with large patches with heavy infection overlapping. Plants in the center of patches have short height and delayed flowering	Important crop damage

Table 3. Sunflowers broomrape seed bank increase in the soil in two and nine year rotation schemas considering, 30.000 seed/shoot, a survival of 90% per year of previous broomrape seed soil stock and 2% of this seed bank infecting sunflowers the next time it is planted.

		Potential		
Rotation cycle	Sunflower crop	Broomrape Shoots/Ha	Broomrape Shoot/plant	Seed produced
2 year rotation	1rst	1	0	30.000
	2nd	486	0,010	14.580.000
	3rd	236.196	5	7.085.880.000
	4th	114.791.256	2.296	3.443.737.680.000
9 year rotation	1rst	1	0	30.000
	2nd	232	0,005	6.973.569
	3rd	54.034	1,1	1.621.022.061
	4th	12.560.343	251	376.810.295.780

Figure 3. Graphic representation of broomrape seed bank increase in 4 consecutive sunflower crops under a 9 year rotation schema considering 30.000 seed/shoot, a survival of 90% per year of previous broomrape seed soil stock and 2% of this seed bank infecting sunflowers the next time it is planted.

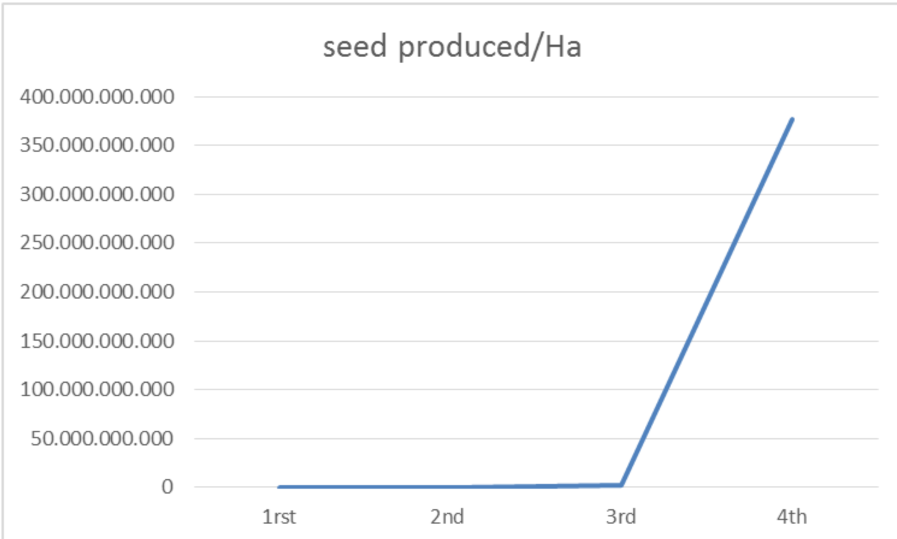


Figure 4. SOLGUARD™® incorporates the good agronomic practices in the integrated broomrape management