Evaluation of an Experimental Stimulant for Germination Induction of Hemp Broomrape (*Orobanche ramosa* L.) in Tobacco Crops (*Nicotiana tabacum* L.)

S. BENVENUTI, A. POMPEIANO, M. MACCHIA, and S. MIELE

Dipartimento di Agronomia e Gestione dell'Agroecosistema, University of Pisa, Italy

Corresponding author: S. Benvenuti, Dipartimento di Agronomia e Gestione dell'Agroecosistema, University of Pisa, Via S. Michele 2, 56124 Pisa, Italy. Tel.: +39 050 599111; fax +39 050 540633, E-mail: Sbenve@agr.unipi.it

Received: 17 April 2003. Accepted: 30 January 2004.

ABSTRACT

BACKGROUND. *Orobanche ramosa* L. (hemp broomrape) is one of the worst weeds affecting tobacco. It causes severe quanti-qualitative damage to production, and selective herbicides for control of this parasite are virtually non-existent. Thus the possibility of combating its development with an innovative stimulant designed to induce prior germination in the field may be of considerable interest.

METHODS. Laboratory and field tests were conducted to test the effect of an experimental germination stimulant (Nijmengen 1 preparation – BASF) on buried seed. Attention focused particularly on seedbank reduction following distribution of the stimulant prior to planting out the crop.

RESULTS. Seedbank reduction was greatest in the upper 10 cm of soil (roughly 75% reduction), but became less marked with increasing seed burial depth. Seedbank reduction resulted in a smaller percentage of parasitized plants and a lesser emergence of spikes. Affected plants showed stunted growth and a lower macronutrient content in the various plant organs.

CONCLUSIONS. The experimental stimulant was effective in achieving some reduction in the seedbank. However, the product is likely to be fully successful agronomically only if applied repeatedly over time, in order to enable it to take effect in the different soil horizons after a number of tillage operations.

Key-words: hemp broomrape *(Orobanche ramosa),* tobacco (*Nicotiana tabacum*), seedbank, weed control, parasitic species.

INTRODUCTION

Orobanche ramosa L. (hemp broomrape) is probably the most damaging weed affecting tobacco (*Nicotiana tabacum* L.), above all in Mediterranean agroecosystems whose pedoclimatic and agronomic conditions are particularly propitious for the biological requirements of this weed species. Thus the Mediterranean Basin countries (above all Spain, Italy, Greece, Israel etc.) are those most frequently infested by *O. ramosa.* Although it can parasitize other crops (such as tomato, potato, sunflower) and even some weeds (Mitich, 1993), it causes the most severe economic losses in tobacco (Sauerborn, 1991). This is due partly to the rigidity of the qualitative standards that must be satisfied by harvested tobacco, and partly also to the elevated commercial value of the tobacco crop.

In Italy the adverse agronomic effects of *O. ramosa* are observed above all in Umbria, where this weed is known by the common name *fiamma* ["flame"]. It is particularly widespread in agroecosystems where extreme simplification of the sequence of crops has frequently involved two-year rotation of tobacco-winter cereals or even, in some cases, tobacco monocrop. Such practices result in accumulation of a large soil seedbank, which often compels farmers to suspend tobacco cultivation for several years since *O. ramosa* is poorly controlled by selective herbicides (Foy and Jacobsohn, 1989; Lolas, 1994). Only glyphosate proves to be effective (Nandula et al., 1999), but its distribution is highly risky for the crop even when treatments are carried out with shelded nozzles. Attempts have been made to induce selectivity by developing transgenic herbicide-resistant crops (Joel et al., 1995), although this proposal is unpopular, particularly in Europe where the use of Genetically Modified Organisms is a source of notable public concern.

A possible alternative strategy may lie in conventional genetic improvement, exploiting the finding that some genotypes of tobacco and al-

so of other crops (Dominguez et al., 1996) show a degree of tolerance (Goldwasseret et al., 2000) or occasionally resistance (Wegman et al., 1991). Such tolerance or resistance is due mainly to mechanical or enzymatic obstacles (Joel et al., 1996) that prevent penetration by the primary haustorium of the parasite.

Another alternative strategy for control of *Orobanche* spp. is to reduce the soil seedbank, as it is known that the larger the seedbank, the greater the risk of crop failure. With seedbanks of autotrophic species, typically observed in tobacco (Miele et al., 2000), the potential weed infestation can be reduced by the widespread practice of "stale seedbed". With *Orobanche,* however, seedbank reduction is difficult to achieve. *Orobanche* seeds exhibit pronounced longevity (Lòpez Granados and Garcìa Torres, 1999); in addition, they remain quiescent in soil in the absence of a parasitizable crop. This is due to the nature of their germination trigger: germination is favored by hormonal substances contained in the seeds themselves (Zehhar et al., 2003), bu it occurs only in presence of root exudates released into the rhizosphere by the host plant (Smith and Van Staden, 1995), and more specifically by the root capillitium (Vail et al., 1990).

Germination induction attempts have also been made with the agronomic strategy of introducing "trap crops" during intercropping periods, so that the trap crop triggers *Orobanche* spp. seed germination (Zonno et al,. 2000). In Umbria, squarrosum clover (*Trifolium squarrosum* Savi) is sometimes used for this purpose, although its agronomic effectiveness is not always appreciable.

Agronomists have thus proposed the adoption of synthesized substances capable of triggering *Orobanche* seed germination, and similar to those produced naturally by the host plant roots. Such products have been under study for several years in the framework of basic research on the biology of *Orobanche* species. One of the most widely used products is GR24 (Johnson, et al., 1981). But so far, most products have proved unable to maintain biological effectiveness in field conditions. In soil, the synthesized molecules are affected by microbiological, chemical and physical alterations that tend to inactivate their activity. Recent research in the chemical industry has now led to an experimental product specifically designed for field application, inasmuch as its chemical-physical properties maintain its efficacy for a period of time sufficient to allow buried seed germination. Products capable of triggering in vitro *Orobanche* seed germination have been available for several years. But the first product designed for field application (and therefore partially resistant to chemical-physical and microbiological alterations) was devised by BASF. This synthesized product (Nijmengen-1 preparation), which is not yet commercially available, contains a cyclohexanone, the active ingredient for the germination trigger. It is mixed with substances capable of protecting it in soil against possible biotic or abiotic alterations.

The purpose of this study was thus to evaluate the agronomic effectiveness of this experimental stimulant distributed in the field. Attention focuses above all on the *O. ramosa* seedbank dynamics, assessing the ability of the product to induce pre-transplantation "suicide" germination, in which weed may not develop because of the absence of any host plant.

MATERIALS AND METHODS

Germination in Petri Dishes

O. ramosa seeds used for the germination trials were collected by hand from the experimental plot during the year preceding the trial (2000). Seeds were cleaned by removal of floral residues and other extraneous material, and stored at roughly 4°C until required for laboratory germination tests in the following spring. Prior to utilization, seeds were soaked in deionized water, in order to achieve the preconditioning known to be necessary for this species (Nun and Mayer, 1993).

Tests were then performed to evaluate the effect on O. ramosa seed germination (without the soil matrix) of the following doses of the experimental product: 0.5, 5 and 50 mg l^{-1}. In some cases fresh seeds (immediately after collection) were used. Five cm diameter Petri dishes, each containing 100 seeds, were used. Dishes were then sealed with parafilm (to maintain humidity) and placed in climatic cabinets set to 20°C, and incubated in darkness. These incubation conditions were shown in previous studies (Kebreab and Murdoch, 1999) to be optimal for

Orobanche spp. seed germination induction. On account of the small size of hemp broomrape seeds, germination was observed by means of an optical microscope (Optec model LF2) allowing 45x magnification. Seeds were considered germinated at the appearance of the primary haustorium. A completely randomized experimental design with 5 replication was adopted.
Both the above tests and the seed analyses described below were carried out at the Analysis and Research Laboratory accredited by ISTA - International Seed Testing Association) of the Dipartimento di Agronomia e Gestione dell'Agroecosistema of the University of Pisa.

Germination in Inspectable Pots

To assess the effectiveness of the experimental product in a typical soil matrix, experiments were conducted in metal pots (20 × 10 cm, depth 30 cm) equipped with special transparent glass inspection windows to allow *in situ* observation of buried seed germination. To facilitate inspection without disturbing the contents, seeds were placed in soil near the inspection windows. Trial seed burial depths were 5, 15 and 25 cm. 100 seeds were placed at each depth. In order to ensure a valid comparison with the field tests, soil taken from the experimental plot was used.
The germination stimulant was distributed on the soil surface (in absence of the plant host) at a concentration of 5 mg l^{-1} (10 ml per pot). Irrigation (500 ml per day) was applied in order to favor diffusion of the product through the soil layers and to ensure appropriate moisture in the substrate. The germination counts were directly carried out (without any disturbance of the seed-soil matrix) by using the above cited transparent windows.

Table 1. Trial soil physical and chemical characteristics

Gravel (> 2mm)	%	traces
Sand	%	36
Silt	%	43
Clay	%	21
Organic matter (Walkley-Blach method)	%	1.44
pH	-	8.1
Total nitrogen (Kjeldal method)	‰	0.8
Assimilable phosphorus (Olsen method)	mg Kg^{-1}	4.5
Assimilable potassium (international method)	mg Kg^{-1}	129
Electric conductivity	µS	179.5
Total limestone	%	4.44

An alternative procedure was also adopted for other pots, which were prepared in the same manner as described above. However experimental product was not applied in these pots; instead, the pots contained tobacco plants (as a control of the natural germination environment), already transplanted and acclimatized for several weeks, possessing a root system spread throughout the profile of the pots.
Five pots were used for each of the three treatments (stimulant on the surface or in depth, tobacco plants). The pots were placed in darkness in climatic cabinets, set to 20°C as described above for the Petri capsules. A completely randomized experimental design was likewise used.

Field Trials

A field experiments was carried out in 2001 at the "Fattoria Autonoma Tabacchi" farm near Città di Castello (Province of Perugia), in the locality Cerbara (43° 27' N, 12° 14' E). An experimental field was chosen that was particularly suitable for the trials, on account of the elevated *Orobanche crenata* infestation recorded in previous years. Soil chemical and physical characteristics of the field, which was completely flat, are shown in Table 1. The area has a fairly mild climate, but with frequent winter frosts, and fairly elevated rainfall concentrated mainly in spring and the fall. Figure 1 shows maximum and minimum temperatures and rainfall during the experimental period.

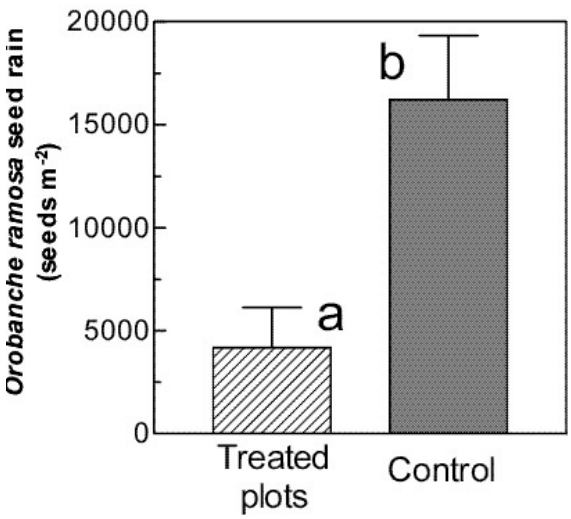

Figure 1. Mean, maximum, and minimum temperature trends of ten-day periods and rainfall during the trial period.

The trial field was divided into 8 plots measuring 900 m^2 (30 × 30 m) each. The germination stimulant was distributed, after main and complementary tillage operations, as a solution (5 mg l^{-1} of the preparation) towards mid-March, at a considerable distance of time before crop transplantation. Adopting a completely randomized block design. The solution was distributed at the rate of 0,1 l m^{-2} on four plots randomly chosen using a conventional sprayer equipped with a 10 m wide bar. All plots (both treated and control) were irrigated several times during the subsequent 2 weeks in order to guarantee the solution penetration in the soil and to ensure the optimal soil moisture required for *Orobanche* seed germination induction.

Table 2 summarizes the agricultural practices utilized in the experiment. Such techniques are representative of the crop management practices adopted in Umbria for production of aromatic tobacco crops such as Virginia Bright.

Seedbank Analysis

Both before (beginning of March) and after distribution of the stimulant (in post-transplant, towards the end of May), soil cores (10 cm long and 4 cm diameter) were sampled by using a metal probe. Cores were taken from the following soil layers: 0-10, 10-20 and 20-30 cm. A total of 360 soil samples were obtained (15 sampling points per plot × 3 depths × 8 plots).

Prior to seed extraction from the cores, the soil samples were pre-treated for roughly 10 hours in a solution containing 5 g^{-1} of sodium hexametaphosphate, in order to disperse the colloid matrix and thus facilitate the subsequent washing stages. Washing procedures adopted a previously described methodology (Benvenuti et al., 2001) using a hydrojet equipped with a pressure valve, so that pressure could be regulated to the minimum required and thus avoid any damage to seeds. A sequence of sieves with different pore size (from 5 to 300 μm) was then used, to eliminate the smaller (silt and clay) as well as the larger coarse sand and gravel) fractions. The *hemp broomrape* seeds were then extracted from the remaining material, which had an exclusively sandy matrix, by allowing them to float in a saturated sodium iodide solution. The latter solution was adopted by virtue of its notable specific weight (roughly 1.7), enabling any seeds present to float on the surface. Seeds were then collected, counted and subjected to the tetrazolium viability test, according to a procedure already utilized for this species (Lòpez Granados and Garcìa Torres, 1999). This technique overcomes the impossibility of dissecting such small seeds by decoloring the integument with sodium hypochlorite, allowing detection of the color of the viable embryo with the aid of the optical microscope. The test was replaced by morphological seed analysis in cases when the primary haustorium had already appeared. Presence of the haustorium indicated that the seeds were no longer viable, as this leads to irreversible germination which would require the germinated seed to parasitize a host plant within no more than a few days in order to survive.

Evaluation of the Infestation and Weed Seed Production

At the end of the crop growth season (mid-September), sampling was carried out to quantify the number of plants affected by the parasite, the number of spikes per host plant and weed seed quantity. Thus a count was taken of af-

Table 2. Agrotechniques utilised for tobacco cultivation during the trial.

Main tillage	Plowing 30 cm
Complementary tillage	2 harrowings
Rotation	Tobacco – winter cereal
Pre-transplantation fertilization	160 Kg ha^{-1} of P_2O_5 and 175 Kg ha^{-1} of K_2O
Top dressing	35-125 Kg ha^{-1} NK as potassium nitrate
Time of transplantation	second half of May
Seedling rearing	floating
Transplantation density	2.2 plants m^{-2} (100 cm x 45 cm)
Cultivar	Virginia Bright var. K394
Irrigation	3,150 m^3 ha^{-1} in 9 operations of 350 m^3 each
Cultural practices	topping (mechanical)+ shoot inhibition (maleic hydrazide)
Chemical herbicide	prior to transplantation (diphenamid 1.2 Kg ha^{-1})
Harvest	Manually three times in the period August-September

fected plants in each of the experimental plots, also recording the number of spikes observed in the vicinity of the host plant. Weed seed production was calculated by collecting a representative sample of 30 inflorescences per plot. These were allowed to dry in the air for roughly 1 month, after which the following seed quantification procedure was conducted: 1) measurement of the weight of seeds produced per inflorescence using a precision scale (Mettler, mod.AE166); 2) calculation of the weight of 1000 seeds according to the internationally approved method (ISTA, 1999); 3) calculation of the total number of seeds produced per inflorescence, using the above parameters. Finally, the quantities calculated were transformed into seeds per surface unit by means of the observed infestation levels.

Observations on Growth and Production

At full crop maturity (end of August), the following crop biometric parameters were measured: dry matter produced by the various plant organs (leaves, stems and roots), height, leaf area (measured by electronic planimeter, model Li-Cor 3000) and the related Leaf Area Index (LAI), marketable yield production. Measurements were carried out in the laboratory on 10 plants from each of the experimental plots, according to a randomized procedure. Plants were then maintained in a ventilated oven at 40°C until the tissues were completely desiccated, for measurement of accumulated dry matter.

Parasitization Kinetics

Throughout the crop growth season, some destructive examinations were performed on tobacco plants in order to observe the parasitization kinetics of *Orobanche ramosa.* Data on the following developmental stages were recorded: formation and swelling of tubercles, emergence of spikes, flowering and seed ripening. As seed germination and penetration of the haustorium could not be detected in the field on account of the small seed size, data obtained in the earlier *in situ* germination experiment with the inspectable pots.

Chemical Analyses on Plant Samples

Chemical analyses performed on plant samples (N, P and K) were carried out according to the standard procedures (Walinga et al., 1995) adopted at the Chemical Agricultural Analysis Laboratory of the Dipartimento di Agronomia e Gestione dell'Agroecosistema.

Statistical Analyses

The experimental data were submitted to analysis of variance (ANOVA) after transformation of percentages into angular values. Significant differences among the means were evaluated using the Student-Newman-Keuls ($p<0.05$ and/or $p<0.01$) test. In some cases linear regressions were performed and then submitted to significance tests. For each statistical analysis, the Costat commercial software was used.

RESULTS AND DISCUSSION

Germination

Figure 2 shows *in vitro O. ramosa* seed germination at increasing concentration of the germination stimulant. In the absence of the product, no germination event was observed, while even at the lowest tested concentration (0.5 mg l^{-1}) almost 20% of seeds showed outgrowth of the primary haustorium. Germination was almost total (roughly 95%) at 5 mg l^{-1}, and since this concentration gave a result statistically not different ($p<0.01$) to that at the highest concentration (50 mg l^{-1}), it is evident that the germination trigger was saturated in both cases. It

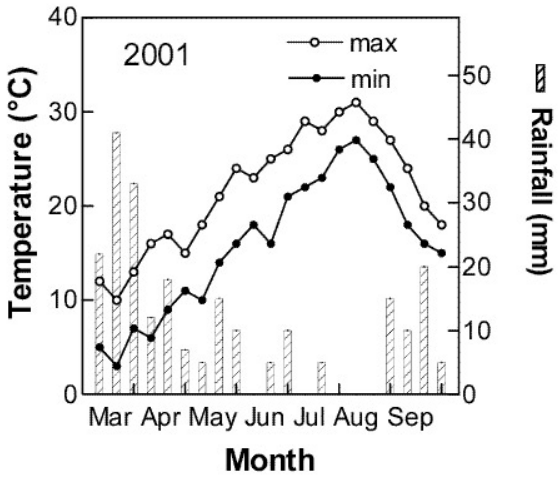

Figure 2. Germination of *Orobanche ramosa* seeds as a function of increasing (from 0 to 50 mg l^{-1}) concentrations of the experimental stimulant (Nijemengen 1 preparation –BASF). Incubation in darkness at 20°C. Means with different letters differed statistically for $p<0.01$ (SNK test).

should be noted that these germinations tests were less effective when performed on "fresh" seeds (where "fresh" is defined as just a few days after seed collection, Benvenuti et al., 2002). This was due to the fact that *Orobanche* spp. seeds are often characterized by primary dormancy (Lòpez Granados et al., 1996). Moreover, in order for seed of this species to be receptive to the biochemical message of the trigger (or, alternatively, of host plant root extract, Restuccia and Mauromicale, 1991) a period of seed preconditioning in a moist environment is required, as pointed out by other Authors (Nun and Mayer, 1993). If such preconditioning is not carried out (data not shown), germination induction is decidedly lower.

Seedbank

Figure 3 shows the seedbank accumulated over time in the various soil horizons prior to the start of the experiments. Over 100,000 seeds m^{-2} were found throughout the entire soil profile examined (0-30 cm), testifying to the frequent infestations affecting the tobacco crops in previous years. The scattering of seed throughout the plowed profile corresponds to findings reported in the literature concerning the pattern of seed distribution following normal agricultural operations of partial inversion of soil horizons (Cousens and Moss, 1990). Such operations tend to distribute seeds roughly half-way down the profile, as highlighted by the fact that roughly 48,000 seeds m^{-2} were found in the intermediate soil tillage profile (10-20 cm) while the shallowest (0-10 cm) layer had a seedbank similar to the deepest (20-30 cm) layer (roughly 28,000 seeds m^{-2}). This large seedbank shows that the survival strategy of this species consists in production of a large quantity of seed (Mitich, 1993) and seed persistence in the field (Lòpez Granados and Garcìa Torres, 1999).

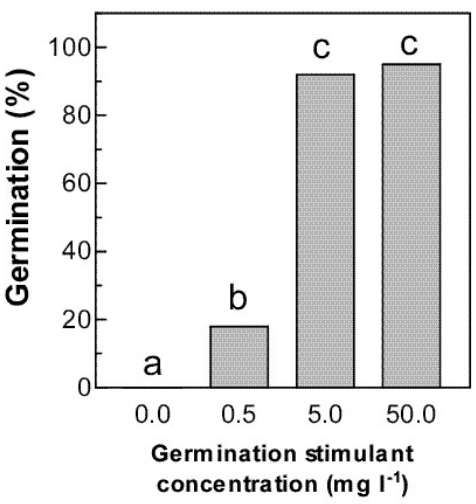

Figure 3. *Orobanche ramosa* seedbank (viable seeds m^{-2}) detected in the various soil profiles of the trial field. Horizontal bars indicate the standard error of the means.

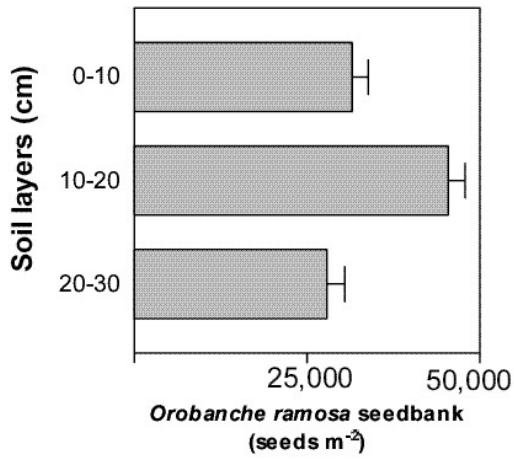

Figure 4. Reduction of the seedbank (shown as viable seeds) in the various soil layers (0-10, 10-20 and 20-30 cm) following distribution of the experimental product prior to crop transplantation. Means with different letters differed statistically for p<0.01.

Figure 4 shows the effect of the stimulant applied in the field, indicating the percent reduction of the seedbank in each of the three above mentioned soil horizons (0-10, 10-20 and 20-30 cm) a few weeks after distribution of the product. Effectiveness was greatest in the upper soil layer, and decreased with increasing depth. Thus in the upper horizon the stimulant induced "suicide germination" in roughly 75% of seeds, but this percentage declined to 25% of seeds in the intermediate horizon, followed by a drastic loss of efficacy (to as little as 3%) in the lowest layer.

It should be noted that the seedbank values shown in Figure 4 refer exclusively to seeds found to be viable according to the tetrazolium test.

The scanty effectiveness of the stimulant in the intermediate and deeper soil layers could be attributed to two factors: either depth-induced germination inhibition as observed in other weed species (Benvenuti et al., 2001), probably

on account of limited oxygen diffusion (Benvenuti and Macchia, 1995), or limitations on penetration of the product into deeper soil horizons.

In Situ Germination

Figure 5 shows *O. ramosa* seed germination at the increasing burial depths to which the stimulant was transported either after surface application of the product or as a result of the presence of host plant roots (tobacco plants). In the former case, product efficacy decreased with increasing depth, mirroring the above-described field observations on its decreasing effectiveness at increasing burial depths. But in the presence of tobacco plant roots, germination was most elevated at the greatest depth, and decreased with shallower burial. This prompts a twofold consideration: 1) under normal field parasitization, with crop presence, the soil horizon most severely affected by hemp broomrape germination was the layer most intensely explored by the root capillitium. That is to say, the most elevated germination percentage occurred in the deeper layer, where the greatest presence of host plant hypogeal organs was found (data not shown); 2) it appears that *O. ramosa* seeds are not inhibited by the increasing oxygen deficiency observed at greater depths (Drew, 1990), suggesting that this species does not conform to normal soil germination ecology. Such a phenomenon could be due to a difference in evolutionary pressure. Thus normal flora have developed a highly sophisticated perception of excessive burial depth, which induces seed dormancy as the endosperm has limited energy resources. In the case of hemp broomrape, the mere presence of host plant root tissue is sufficient for germination since the delicate heterotrophic stage terminates with parasitization and not with seedling emergence.

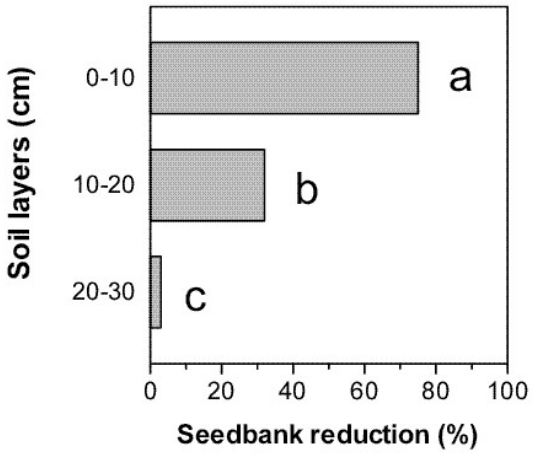

Figure 5. Germination in inspectable pots of *Orobanche ramosa* seeds buried at increasing depth (5, 15 and 25 cm), stimulated (black-tilled dots) by the experimental stimulant or in presence of tobacco plant roots (empthy dots). Both linear regressions were significant for p<0.05.

Overall, however, a clear pattern of declining efficacy linked to a limited ability of the stimulant to penetrate into deeper soil layers can be seen. This pattern is further confirmed by observations on the inspectable pots. When the stimulant was applied through the openable windows directly on buried seeds, germination was elevated (data not shown) independently from depth of burial. On the other hand, in the field the stimulant showed a lower activity than the *in vitro* tests. Thus as compared to the almost 100% germination obtained in Petri dishes, percentages were practically halved even in cases of greatest biological effectiveness such as direct distribution of the stimulant on buried seed.

Parasitization in the Field

The percentage of plants affected by *O. ramosa* in the presence or absence (control) of seedbank treatment for "suicide germination" induction is shown in Figure 6A. The decrease in seed viability allowed a significant ($p<0.01$) reduction in number of parasitized plants, with only about 5% of plants being affected after pretransplantation soil treatment as compared to almost twice as many (9%) among controls. This result confirms the usefulness of the experimental product, even though it is clear that a single application cannot provide a definitive solution given that, as suggested by the seedbank analysis, many seeds, especially those situated at greater burial depth, are not influenced by the biological action of the germination trigger. The seedbank reduction led to a decrease in the number of emerged spikes per host plant, from roughly 12% (control) to about 5% (Figure 6B). This does not necessarily imply an appreciable decrease in agronomic damage to the crop. It has been reported (Hibberd et al., 1998) that impairment of photosynthetic activity and the resulting yield decrease of individual host plants are not related to the number of weed

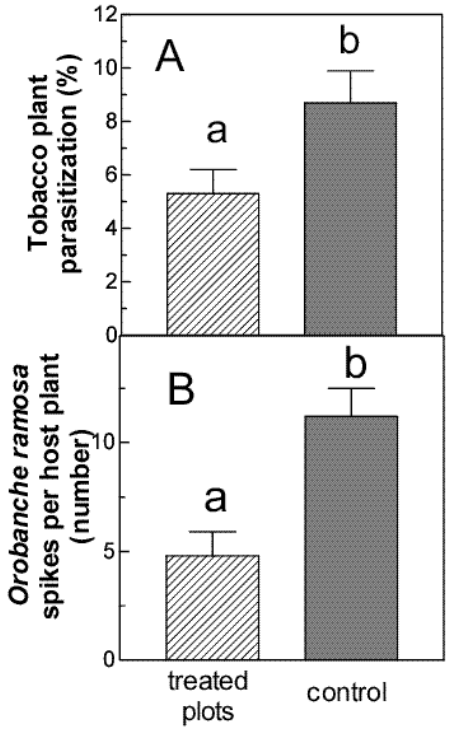

Figure 6. (A) Tobacco plants affected by *Orobanche ramosa* following application of the experimental product. (B) *Orobanche ramosa* spikes for each of the plants affected by the parasite following application of the experimental product. Means (standard error indicated by vertical bars) with different letters differed statistically for p<0.05 (SNK test).

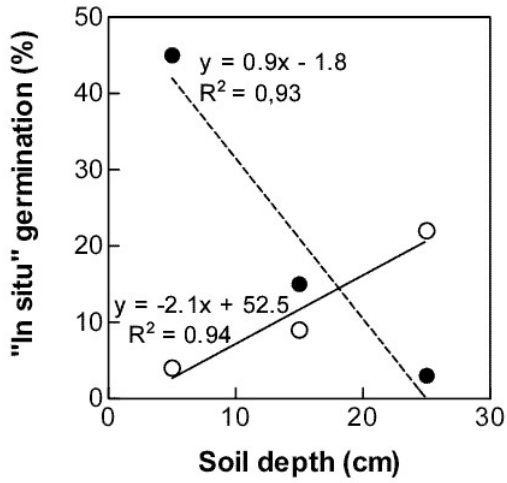

Figure 7. Diagram of *Orobanche ramosa* parasitization kinetics in tobacco.

seedlings. In other words, damage is already saturated by the presence of a single weed, probably on account of "weed-weed" competition.

Parasitisation Trend

Figure 7 presents a diagram of the parasitization trend observed during the various crop phenological stages. Roughly 2-3 weeks after the crop was planted out (end of May), the quiescent seedbank situated in the immediate vicinity (only a few mm) of the host plant roots gave rise to germination and outgrowth of the primary haustorium. This phenomenon is guided by chemiotropism (Joel et al., 1994), which has been shown to be essential in order for the haustorium to reach the host plant tissues. Since it was impossible to detect these early parasitization stages in the field (as pointed out in the Material and Methods section), several tobacco plantlets were grown in the inspectable pots described earlier, with the aim of closer inspection of the parasitization. For subsequent stages, field determinations revealed a prolonged phase of tubercle swelling (initially star shaped) which extended until the middle of August, for a total duration of over 2 months. This phase is known to involve maximum subtraction of nutrient substances (Press, 1995), and it is precisely their accumulation in the parasite that gives rise to tubercle swelling. After this phase, spike emergence was observed. This was a scalar phenomenon as new spikes continued to emerge throughout September. Inflorescences appeared on the spikes as early as a few days after emergence, i.e. from the end of August onwards. Physiological seed ripening, which took place fairly rapidly – within 2 weeks - began in mid-September and then extended into subsequent periods, depending on time of emergence and flowering. Overall, the *O. ramosa* cycle as observed in the Umbrian agroecosystem had a duration of about 4 months.

Host Plant Growth

Growth parameters for above-ground (leaf and stem dry matter) and below-ground (root system dry matter) biomass and some biometric parameters (height, leaf area and LAI) recorded on parasitized and unparasitized (control) plants are shown in Figure 8. Except for the stem (Fig. 8B), host plant biomass was affected by parasitization ($p<0,05$): the leaf apparatus was practically halved as compared the control (80 g , Fig. 8A), and the root system was reduced from 50g (control) to 35 g (Fig. 8C).

Height (Fig. 8D) also decreased from roughly 105 cm in control plants to less than 70 cm in presence of the weed parasite. In effect, this marked difference in height acted as a diagnostic signal of the presence of the parasite, prompting the monitoring of spike growth, which was carried out by destructive investigations on affected plants. Leaf area (Fig. 8E) and leaf area index (Fig. 8F) were drastically reduced as a result of parasitization.

The host-parasite interaction also interfered with the macronutrients N, P, and K, so that the crop was not only quantitatively but also qualitatively impaired. Thus Table 3 shows that all organs of affected plants exhibited significant ($p<0.05$) reductions in nutrient elements in the analyzed tissues. The resulting nutritional deficiencies, in particular nitrogen deficiency, cause the leaf yellowing that is typically observed in parasitized plants, due to impaired chlorophyll synthesis (Ernst, 1986).

Table 3. Chemical analyses performed on leaf, stem and root dry matter of tobacco plants reared in the presence or absence (control) of *Orobanche ramosa* parasitization. In each case, the paired data (parasitized and control) for each nutrient analysed in the various plant organs differed statistically for $p<0.05$.

Plant part	Growth conditions	Macronutrients analyzed N	P	K
leaves	parasitized	1.70	1.93	1.21
	control	1.82	1.82	1.37
stem	parasitized	0.98	1.38	1.24
	control	1.13	1.79	1.49
roots	parasitized	1.09	0.15	0.79
	control	1.14	0.18	0.85

Crop Yield

Figure 9 shows production in the treated and untreated (control) plots. Despite the favorable results of the present experiment, no appreciable differences in production were obtained, due to the large initial seedbank (over 100,000 seeds m^{-2}). Treated plots achieved only 10 % greater production, which was not statistically significant ($p<0,05$) compared to control. This clearly shows that in cases of heavy weed infestation, a single treatment is not sufficient for preventive defense of the crop. Although the stimulant tested in this trial has marked biological activity, it is likely to achieve satisfactory agronomic effectiveness only if application is repeated over time. This inference is parallel to the well-known fact that the soil seedbank represents the effects of long-term accumulation of weed seeds deriving from prior growth seasons (Benvenuti, 1995), and its elimination is also a long-term process. This is particularly true in the case of *Orobanche,* whose survival strategy is based on seeds endowed with elevated dormancy and longevity

Weed Seed Production

The quantity of seed produced by *O. ramosa* is shown in Figure 10. Considerably lower seed dispersal was detected in the treated plots (roughly 10,000 seeds m^{-2}) in comparison to control plots (roughly 4,000 and 17,000 seeds

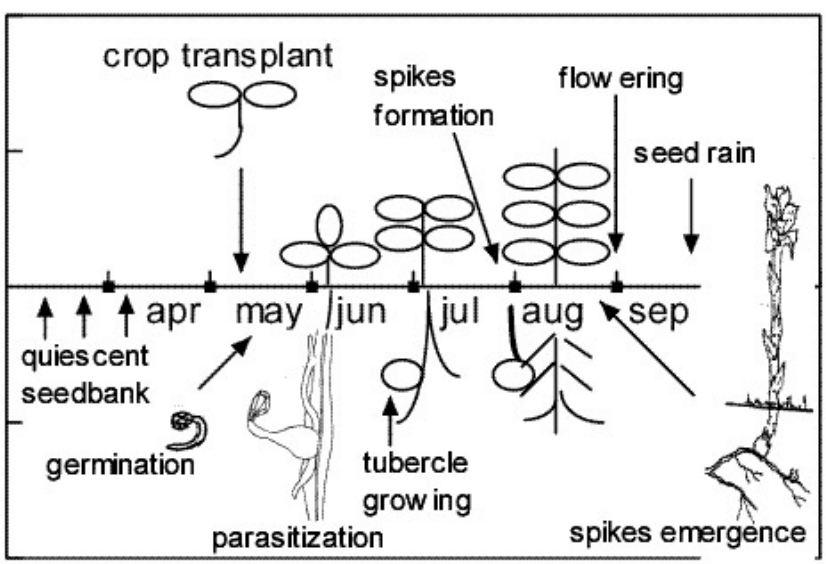

Figure 8. Growth parameters of tobacco plants affected by the parasite: leaf (A), stem (B) and root (C) dry matter; plant height (D); leaf area (E) and Leaf Area Index (F). Means (standard error indicated by vertical bars) with different letters differed statistically for $p<0.05$.

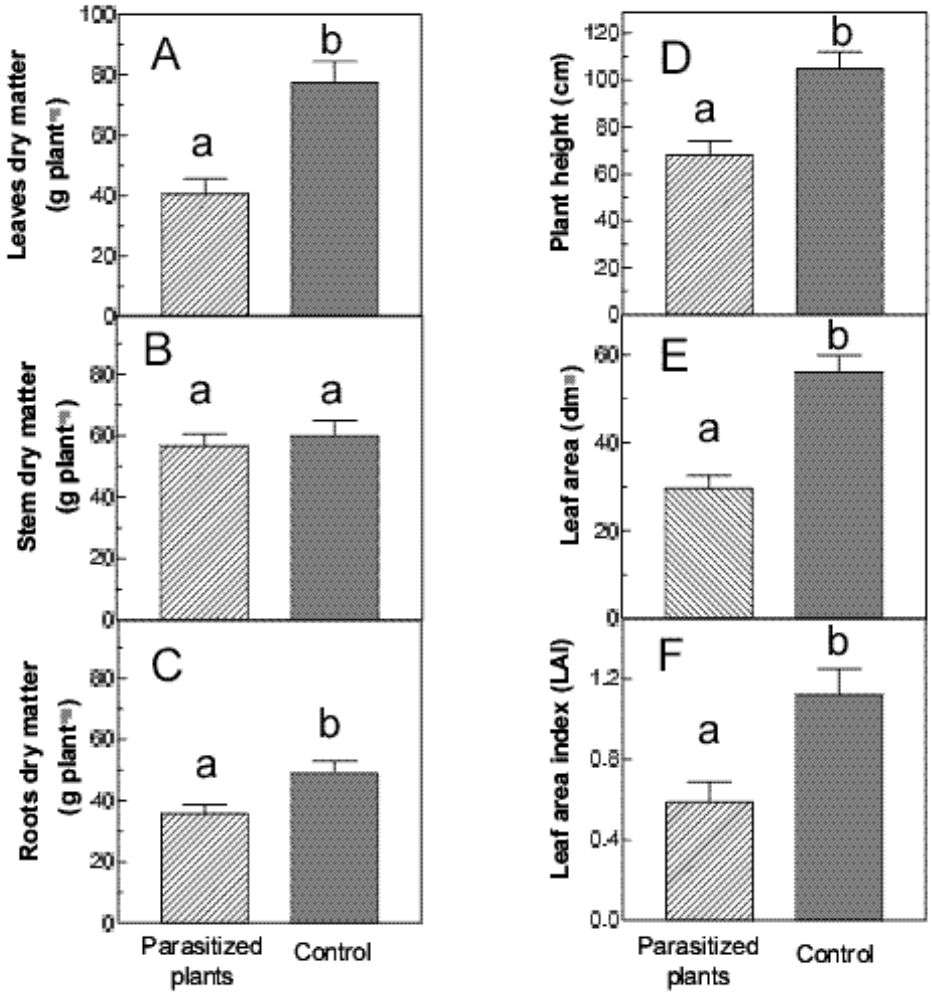

Figure 9. Tobacco yield (expressed as leaf dry matter in t ha^{-1}) in plots treated with the experimental stimulant. Means (standard error indicated by vertical bars) with different letters did not differ statistically for p<0.05 (SNK test).

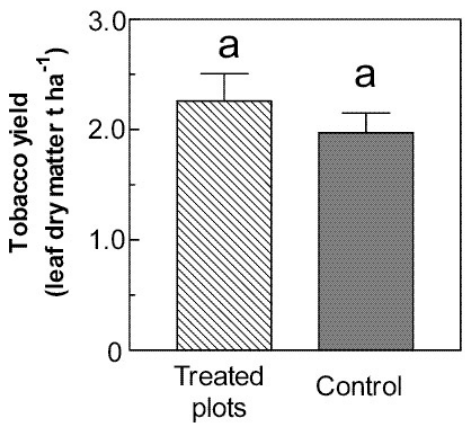

Figure 10. Seed production of *Orobanche ramosa* infestation in treated and untreated (control) plots. Means (standard error indicated by vertical bars) with different letters differed statistically for p<0.05 (SNK test).

m^{-2} respectively). This was due to the twofold effect (mentioned above in connection with Figure 6) of a smaller number of affected plants per surface unit and a smaller quantity of emerged spikes per parasitized plant. In terms of long term forecasting of weed dynamics, these results should lead to lower levels of weed seed production. This favorable prospect should be taken into consideration in agronomic evaluation of the test product. Furthermore, the elevated quantities of annually produced seed could be treated with the germination stimulant prior to burial, in order to counteract the tendency of the soil matrix to limit its biological efficacy. In other words, although some of the newly produced seeds are endowed with primary dormancy, it is still feasible to suggest in-

ducing suicide germination prior to seed *incorporation in the soil horizons from which parasitic weeds are known to emerge.*

CONCLUSIONS

The pronounced *in vitro* biological efficacy of the germination stimulant was maintained in soil, although it was partially attenuated by the limited penetration of the product into the circulating soil solution. Thus the deepest seedbank remained little affected by the stimulant. However, the upper soil layer (0-10 cm) showed a very marked depletion of viable seeds following application of the germination trigger, leading to an appreciable reduction in number of plants affected and number of individuals per host plant. But this was not paralleled by an appreciable reduction in agronomic damage to the crop, because of the very large initial seedbank. It should also be kept in mind that the presence of even a single parasite on the host plant is capable of inflicting maximum damage on the host, as reported in the literature (Hibberdet al., 1998). Therefore, greater success in preventive control of *O. ramosa* could perhaps be obtained by a second application of the product, to be carried out after the crop harvest when annual seed production has been completed. Thus the spring application would be applied to combat the buried seeds, while the fall application would be active against seed disseminated on the soil surface. This strategy could probably achieve a substantial reduction of the seedbank. Finally, periodic monitoring of the *Orobanche* spp. seedbank in order to assess the efficacy of treatments could provide useful data for prediction of future weed dynamics, as already proposed for other crops (Bernhard et al., 1998). This would make it possible to estimate in advance whether the economically viable threshold for planting a tobacco crop can be reached. Agronomic failures caused by excessive weed infestation could be averted, by establishing alternative crops not susceptible to this parasite.

REFERENCES

Benvenuti S., 1995. Ecologia della “seed bank”: aspetti fisiologici ed implicazioni agronomiche nella gestione della flora avventizia. Riv. Agron., 3, 204-219

Benvenuti S., Macchia M., 1995. Hypoxia effect on buried weed seed germination. Weed Res., 35, 343-351.

Benvenuti S., Macchia M., Miele S., 2001. Quantitative analysis of buried weed seedling emergence with increasing soil depth. Weed Sci., 49, 528-535.

Benvenuti S., Pompeiano A., Macchia M., Miele S., 2002. *Orobanche* seed bank dynamics in tobacco by using a germination stimulant. Proc. 12th EWRS Symposium, 380-381. 24-27 July, Wageningen, The Netherlands.

Bernhard R.H., Jensen J.E., Andreasen C., 1998. Prediction of yield loss caused by *Orobanche* spp. in carrot and pea crops based on the soil seedbank. Weed Res., 38, 191-197.

Cousens R., Moss R.S., 1990. A model of the effects of cultivation on the vertical distribution of weed seeds within the soil. Weed Res., 30, 61-70.

Dominguez J., Melero-Vara J.M., Ruso J., Miller J. and Fernandez-Martinez J.M., 1996. Screening for resistence to broomrape (*Orobanche cernua*) in cultivated sunflower. Plant Breeding, 115, 201-202.

Drew M.C., 1990. Sensing soil oxygen. Plant Cell and Environment, 13, 681-693.

Ernst W.H.O., 1986. Mineral nutrition of *Nicotiana tabacum* cv Bursana during infection by *Orobanche ramosa*. Proc. Workshop on Biology and Control of Orobanche, 67-70. LH/VPO Wageningen, Holland.

Foy C.L., Jacobsohn J.R., 1989. Recent approaches for chemical control of broomrape (*Orobanche* spp.). Rev. Weed Sci., 4, 123-152.

Goldwasser Y., Plakhine D., Yoder J.Y., 2000. *Arabidopsis thaliana* susceptibility to *Orobanche* spp. Weed Sci., 48, 342-346.

Hibberd J.M., Quick W.P., Press M.C., Sholes J.D., 1998. Can source-sink relations explain responses of tobacco to infection by the root holoparasitic angiosperm *Orobanche cernua*? Plant Cell Environ., 21, 333-340.

ISTA, 1999. International rules for seed testing. Seed Sci. Technol., 27 (suppl.), 50-52.

Joel D.M., Losner-Goshen D., 1994. The attachment organ of the parasitic angiosperms *Orobanche cumana* and *O.aegyptica* and its development. Can. J. Bot., 72, 564-574.

Joel D.M., Portnoy V.H., 1996. The angiospermous root parasite *Orobanche* L. (*Orobancaceae*) induces expression of pathogenesis related (PR) gene in susceptible tobacco roots. Ann. Bot., 82, 515-518.

Joel D.M., Kleifeld. Y., Losner-Goshen D., Herzlinger G., Gressel J., 1995. Transgenic crops to fight parasitic weeds. Nature, 374, 220-221.

Johnson A.W., Gowdy G., Hassanali A., 1981. The preparation of synthetic analogues of strigol. J. Chem. Soc. Perkin Transaction, 1, 1734-1743.

Lolas P.C., 1994. Herbicides for control of broomrape (*Orobanche ramosa* L.) in tobacco (*Nicotiana tabacum* L.). Weed Res., 34, 205-209.

Lòpez Granados F. e Garcìa-Torres L., 1996. Effects of

environmental factors on dormancy and germination of crenate broomrape (*Orobanche crenata* Forsk.). Weed Sci., 44, 284-289.

Lòpez Granados F., Garcìa Torres R., 1999. Longevity of crenate broomrape (*Orobanche crenata*) seed under soil and laboratory conditions. Weed Sci., 47, 161-166.

Kebreab E., Murdoch A., 1999. A model of the effects of a wide range of constant and alternating temperatures on seed germination of four *Orobanche* species. Ann. Bot., 84, 549-557.

Miele S., Macchia M., Benvenuti S., 2000. Soil seed bank evaluation for weed control strategy in tobacco: first results of a European Community funded project. III International Weed Science Congress, 133-134. Foz do Iguassu, Brazil, 6-11 June.

Mitich, L.W., 1993. *Orobanche* - the broomrapes. Weed Technol., 7, 532-535.

Nandula V.K., Foy C.L., Orcutt D.M., 1999. Glyphosate for *Orobanche aegyptica* control in *Vicia sativa* and *Brassica napus*. Weed Sci., 47, 486-491.

Nun N.B., Mayer A.M., 1993. Preconditioning and germination of *Orobanche* seeds: respiration and protein synthesis. Phytochem., 34, 39-45.

Press M.C., 1995. How do the parasitic weeds *Striga* and *Orobanche* influence host carbon relations? Asp. Appl. Biol., 42, 63-70.

Restuccia G., Mauromicale G., 1991. Influenza di estratti radicali di alcune leguminose da granella sulla germinazione del seme di *Orobanche crenata* Forsk. Riv. Agron., 25, 422-426.

Sauerborn J., 1991. The economic importance of the phytoparasites *Orobanche* and *Striga*. In: Ramson J.K., Musselmann L.J., Worsham A.D., Parker C. (eds.): Proc. 5th International Symposium on parasitic weeds, 137-143. Nairobi, Kenya.

Smith M.T., Van Staden J., 1995. Infochemical: seed.fungus-root continuum. A review. Environ. Exp. Bot., 35, 113-123.

Vail S.L., Dailey O.D., Blanchard E.J., Pepperman A.B., Riopel J.L., 1990. Terpenoid precursors of strigol as a seed germination stimulants of broomrape (*Orobanche ramosa*) and witchweed (*Striga asiatica*). J Plant Reg., 9, 77-83.

Walinga I., Van der Lee J.J., Houba V.J.G., Van Vark W., Novazamsky I., 1995. Plant Analysis Manual. Kluwier Academic Publishers, Londra.

Wegman K., von Elert E., Harloff H.J., Stadler M., 1991. Tolerance and resistence to *Orobanche*. In: Wegmann K., Musselman L.J. (eds.): Progress in *Orobanche* Research, 318-321. Eberhard-Karls-Universität, Tübingen, Germania.

Zehhar N., Ingouff M, Bouya D., Fer A., 2003. Possible involvement of gibberellins and ethylene in *Orobanche ramosa* germination. Weed Res., 42, 464-469.

Zonno M.C., Montemurro P., Vurro M., 2000. *Orobanche ramosa*, un'infestante parassita in espansione nell'Italia meridionale. Inf. Fitopat., 4, 13, 21.

VALUTAZIONE DI UN PRODOTTO SPERIMENTALE PER LA GERMINAZIONE DI *OROBANCHE RAMOSA* L. NELLA COLTURA DI TABACCO (*Nicotiana tabacum* L.)

SCOPO. *Orobanche ramosa* è una delle più importanti avversità biotiche del tabacco a causa sia degli elevati danni quanti-qualitativi sulla produzione che per la pressochè assenza di erbicidi selettivi per il suo controllo. La possibilità di contrastare la sua dinamica di sviluppo con un prodotto concepito per l'induzione germinativa in campo può risultare di estremo interesse per la gestione di questa specie.
METODI. Sono stati effettuati esperimenti di laboratorio e di campo per testare l'effetto sui semi interrati di un prodotto sperimentale, concepito per stimolare la loro germinazione. In particolare è stata esaminata la riduzione della seedbank in seguito alla distribuzione del prodotto in pre-trapianto.
RISULTATI. L'impoverimento di semi, risultato massimo nei 10 cm più superficiali (circa il 75% di riduzione), ma è risultato inversamente proporzionale all'aumento del loro grado di interramento. Tale riduzione, ha comportato una minor percentuale di piante parassitizzate ed una minore emergenza di turioni. Le piante colpite hanno mostrato sia evidenti contrazioni di crescita che una minore presenza di macronutrienti nei vari organi della pianta.
CONCLUSIONI. Il prodotto sperimentale appare di indubbia efficacia nella riduzione nella seedbank. Tuttavia il suo successo agronomico appare legato ad un suo uso ripetuto nel tempo in modo da poter consentire al prodotto di agire, dopo i vari interventi di lavorazione, nei vari orizzonti di suolo.

Parole chiave: : *Orobanche ramosa,* tabacco (*Nicotiana tabacum*), seedbank, controllo infestanti, specie parassita