

Screening of crop germplasms for insect-pests

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ABSTRACT

Common methods to screen crop germplasms for insect-pests in the fields require sampling techniques, observation of insect populations, evaluation of insect-pest development and evaluation of crop-germplasms resistance mechanisms to herbivorous insects. This paper describes the most used simple methods and tests for screening the crop-resistant germplasms for insect-pests, with special reference to aphids. This paper serves as manual for easy use in field by students and young researchers, while primary observations and experiments. It includes, the insect sampling techniques (counting on plants, trapping, extraction from plants), observations in the fields (abundance on plants and soil, eggs laying), evaluation of insect population (under natural and artificial infestation, population parameters) and evaluation of resistance mechanisms (antixenosis, antibiosis, tolerance).

Key words: Abundance, antibiosis, antixenosis, crop-germplasms, insects, pests, population tests, resistance mechanisms, screening, tolerance

1. INTRODUCTION

Phytophagous insects cause significant crop losses and decreases the yield quality even at moderate pest level (10). The plant damage depends on insect species, its developmental stage and type of the mouthparts, and chewing pests are more harmful than suckers (75). Thus effective, precise and environmentally friendly modern plant protection methods need to be developed to control the pest populations (58,153). The insect pests perform differently on crops and in natural landscapes (mixed flora). For example, the aphid population is much bigger on the field edges than in centre of field. On the field edges, higher contamination of *alate* and/or *apterous* insect-pests from other host-plants is found (87). Among the cereal aphids, the grain-aphid, *Sitobion avenae* F. is dominant on wheat, rye and triticale, the bird cherry-oat aphid, *Rhopalosiphum padi* L. occurs on wheat, oat and barley, while the rose-grain aphid, *Metopolophium dirhodum* W. infests the maize plants.

The cultivated crops are genetically uniform. Some plants on the same field, are more heavily infested than others (91). Usually the heaviest infestation occurs on the largest or the tallest plants, and the insect-pest distribution on the crops is not uniform. Many species of phytophagous insects prefer to feed on young or senescent than on mature leaves (70,71). If more than one pest species colonize the same host-plant, their distribution on infested plants can vary considerably. For example, among the cereal aphids, the grain-aphid prefers flag leaves and after heading moves on ears, the bird cherry-oat aphid stays mostly on stems and lower leaves, instead the rose-grain aphid always feeds on lower leaves. The sucking-piercing insects may be found on the upper leaf surface as well on the lower one (150). Quite often it is part of their feeding behavior *e.g.* the walnut aphid, *Panaphis (Callaphis) juglandis* (Goeze) feeds on the upper leaf surface, instead another species of the walnut aphids, *Chromaphis juglandicola* (Kalt.) always occurring on the bottom side of the walnut leaves. Thus it is essential that the screening techniques, used to determine the plant-pest interactions, allow to measure precisely such relationships (57,97).

2. FIELD STUDY OF INSECT-PESTS

Laboratory experiments for screening of crop germplasms for insect-pests produce detail and replicable results but they always raise the question. Do the pests really behave in the same way in the field conditions? This can only be answered by field studies. Field observations may help to determine (i) whether the insects actively orientate to the host and after locating the tested crop-germplasm and (ii) how the pests examine its surface, make a taste bite, or feed briefly and then reject the food or feed continuously? Such observations also help to indicate the use of specific plant cues *e.g.* visual or volatile cues during orientation and tactile or gustatory cues during examining (38).

Work with insects in the field is very difficult since many abiotic and biotic factors influence their populations (82, 84). Thus no one method can be used for the all interactions between the insect-pests and their host-plants. It is also important to understand, when planning the field observations or experiments, that the time taken to make counts increases as the season advances, for the plant surface and the pest population

increase. Therefore the techniques used in study of insect-pest interactions in the field require a good sampling and every effort must be made to ensure that sampling is free from bias. Usually, a fixed number of spaces are made diagonally across the field before taking the nearest plant, or randomly selected measurements should be taken along predetermined rows of the crops. The standard error of the insect counts should be stated, although it is not measure of sampling efficiency.

2.1. Sampling techniques

Choice of sampling techniques depends much on the fact that, if the host-plant can be destroyed/damaged or not during the insect counts. The observer has to remember that removing a plant from a field can profoundly affect the insect population of neighboring plants. When the plots are very small it may mean that the sampling becomes an important factor in growth and further development of plant and pest populations. Thus counting *in situ* is preferred to removing plants or parts of plants from the field (102, 135).

The plant collection in such experiments usually occurs only when there is a large number of insects on plants and it is better to check samples carefully in the laboratory. In most experiments the samples are of fixed sizes *e.g.* check of 100 plants or 10 meters long, with a proper number of replicates. When the leaves are inspected, they usually vary from 100 to a large number and quite often the random choice considers the upper, middle and lower leaf zones of the crops (1,138).

Observers use a standard record card to make the sampling records in the field much easier. They allow to properly place the data to be later analyzed by a computer without any further treatment or rearrangement. During the visit to the field, observer should record as much as possible useful informations such as: side or experimental details, the date, time the record was made, temperature and humidity. Some notes considering the studied crop-plant *e.g.* cultivar name, growth stage, plant age and height, number of plants per row or per unit area, number of leaves per plant and whether plants are healthy or diseased are also suggested (31). In general, the important aspects of insect sampling in the field are: (i). Counting *in situ*, (ii). Extraction from plant material and (iii). Insect trapping in plantation.

2.1.1. Counting on plants

While sampling of insect-pests in the observer records the level of infestation *in situ* counting of insect number on randomly chosen plants. When there is a large or small number of insects, use of infestation scales is preferred (45, 146). The infestation scales are based on classifications that consider grouping the insects into specific categories *e.g.* None (zero), Extremely light infestation, Very light infestation, Light infestation, Medium infestation and Heavily infested plants (5,145). Direct estimation of pest population is done by counting the insects per plant, per unit area of plant or area of examined land. Other suggested techniques are based on the set length of row, number of plants per unit length of row, where the row spacing should be always stated. The insect number per plant can be counted only when the plants are small, or there are a few insects per plant. When a large number of pests occur on infested plants only parts of the plants are usually checked. Such cases require to publish the details about how the observer selected the samples when counted insects on certain part of plants of the tested crop-germplasms. During field

observations, modern techniques such as videos and/or digital camera can be used to record the insect-pests occurring on host-plants.

2.1.2. Insect trapping and extraction from plants

Insect sampling methods in field conditions can be divided into two : (i) Intercept flying insects and (ii) Sample insects from the tested crop-germplasms or from the ground under them (98,106). Large insect populations occurring on plants are very difficult to be directly inspected in the field, thus quite often samples of a fixed number of leaves, stems, all plants or known weights of plant material are collected into plastic bags and then checked in the laboratory. Samples of insect-pests from big trees or shrubs might be collected by shaking and beating infested plants while holding a light-colored tray beneath them. In case of mobile insects, the pitfall traps or counting in quadrates left in the field might be used (136). Other techniques of insect flight' monitoring and collection from crops used in the field are: (i). Radar monitoring (120), (ii). Pheromone traps (125,128) and (iii). Sweep-nets. The sweeps must be made in undisturbed vegetation by the same person and each sample unit consists of exactly determined number of semi-circular sweeps *e.g.* 25 per sample (36,98,124). When the more sophisticated methods of sampling are required the suction techniques may be used. However, the suction samplers have some advantages and disadvantages, only small area is sampled and their activity is pretty much depending on the power of fan (17,67,154). Less used techniques for estimation of insect-pests on crop plants are trapping methods. The filter traps collect air and filter the insects from it, and volume of the air and level over the vegetation is usually controlled (66,94). The impaction traps are coloured for insect attraction, they also may retain the insects with help of grease (sticky traps) (23,26,53,74). There are also water traps containing detergents (41,42,104) and insect light traps stimulating the insect attraction (49, 100, 121).

In laboratory the insect-pests are first extracted from the collected plants and counted using various counting techniques. The most common extraction methods are : (i) knock-down (145); (ii) floating, (iii) washing and filtering techniques (56); (iv) brushing (58,88) and (v) imprinting (144). In counting techniques one can distinguish: volumetric methods [when large number of insects occur on plants (5,73)], counting grids [(when the insects' are separated into different developmental stages (137), electronic counting (88)], and egg counting techniques [when the egg-laying study is done (25,96)].

3. INSECT POPULATION IN FIELD

Screening procedures for crop-germplasms are difficult in field and, hence are first used in glasshouse conditions, but the final verification of screening must be done in field (147). Crop cultivars support pest populations of different size and in different ways and quite often the herbivore populations are also distributed differently (150). However, there are cultivars more suitable (susceptible) and less suitable (resistant) for the insect-pests (31,32). When the pests produce biotypes, races or clones their distribution on the host-plants is far more complicated. The major screening techniques applied in the field are based on various aspects of insect population study, including observation in the open field and on isolated (caged) plants. The study in the open field considers the following

aspects: (i) Pest abundance on plants of tested crop-germplasms, involving insect density, cumulative index of infestation, percentage of infested plants, infestation scales, insect aggregation on tested germplasms, size of colony, pest population structure, population dynamics, clonal variability and duration of pest occurrence on plants; (ii) Eggs' laying (oviposition); (iii) Number of insects on soil in relation to their density on plants (84,101).

3.1. Abundance on plants

Number of insects on the crops in the open field condition refers to effect of host-plant, weather (temperature, wind, rainfall), parasites, predators and fungus on the examined pest population (6,29,39). Such observations compares different crop-germplasms and show if there are any allelopathic interactions. They might be performed on small plots (1m x 1m), bigger observation fields *e.g.* 20m x 5 m, or in the regular production fields, where the observation area is generated (Fig. 1). If different crop-germplasms are planted in the observation field or plots closely located, there are similar conditions to compare their effect on the monitored insect-pests.

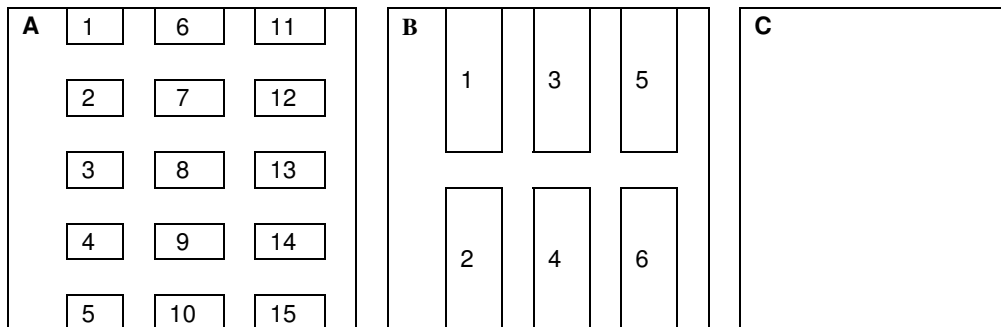


Figure 1. Scheme of observation fields for screening of crop-germplasms to insect pests. A: Small plots, B : Bigger observation fields, C : Productive field

Observation 1. Density on plants

The insect-pest density on the host-plants is first parameter of crop-germplasms screening in field conditions. In general, the higher number (density) of insect-pests occurs on more susceptible crop-germplasms (80,81,83,126).

Materials and observation requirements

Seeds of test crop germplasms, observation field, sowing equipments, fertilizers, record cards, ball pen/pencil.

Observation procedure

Before starting the observations, the field has to be prepared according to the standard requirements for the particular crop. When more than one crop-germplasms are tested, the observation area is divided into smaller parts and seeds of the test germplasms are sown. After the insect-pests arrival, the observer starts to count their numbers on randomly selected plants. The counting procedures has to be adjusted to the required

standard for the certain crops and pests. The most popular are counts of insect pests on: (i). 25-100 plants, leaves or stems randomly chosen across the field; (ii). 25-100 plants, chosen at random in rows of lengths 20 to 30 m inside the field; and (iii). 25-100 plants chosen while the serpentine route in the field (Fig. 2). Counts are made weakly or twice weakly from the pest arrival until the end of its occurrence on the host-plants, using the earlier prepared record cards (Fig. 3). The observation has to be performed in a proper number of replicates and plants selected for infestation assessment must be selected randomly (38,64,152).

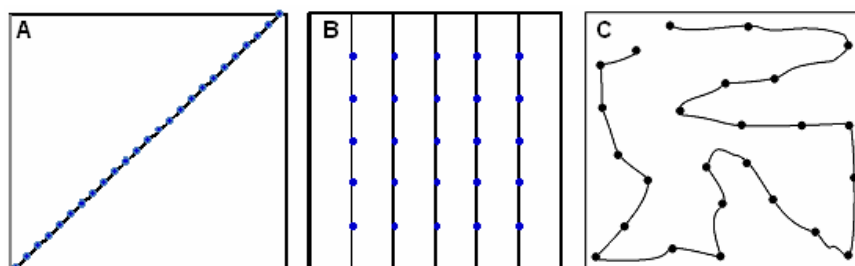


Figure 2. Organization scheme of observation routes. A: Randomly chosen across the field; B: Plants chosen randomly in rows; C: Plants chosen based on serpentine route.

Record card

Name of Crop-germplasm:

Field number:

Date of observation:

Plant number	Pest developmental stage			Plant number	Pest developmental stage		
	Alate	Apterae	Larvae		Alate	Apterae	Larvae
1				11			
2				12			
3				13			
4				14			
5				15			
6				16			
7				17			
8				18			
9				19			
10				20			

Additional notes:

Figure 3. An example of Record Card (Alate : Adult winged females; Apterae : Adult wingless females)

Calculations

Step 1. Population size

When the observation is finished the insect number from all inspections is summarized for each tested crop-germplasm and can be expressed in following ways: (a)

total insect number on the monitored number of plants, (b) an average insect number per plant, during the all season, (c) insect number per plant during the specific plant growth stage and (d) insect number per monitored area of observation field (15). Whole-plot scores should be made independently at least in 3-4 replicates, their results combined and analysis of variance performed. Size of insect population is usually expressed as an average of whole-plot scores for the tested crop-germplasms.

Step 2. Cumulative index of infestation

Another method to express the insect-pest abundance on the tested crop-germplasms is a cumulative index of infestation unit days (149):

$$CII = \sum_{n=1}^{n=k-1} \left(\frac{t_n}{2} \times (x_n + x_{n+1}) \right)$$

Where, n : Number of occasions on which the insects were counted, x_n, x_{n+1} : Consecutive indices; t : Time in days between the consecutive indices x_n, x_{n+1}

For more sophisticated observation and insect counting's, FASA index may be calculated as Under (92).

Step 3. Infestation scales

While the pest population outbreaks or during massive insect attacks, the infestation scales might be used. An universal scale for plants attacked by pests was proposed by Banks (5) and further improved by Way and Heathcote (145). It consists of following degrees of infestation: 0 : None insect on the checked plants, 1 : Extremely light infestation, 2 : Very light infestation, 3 : Light infestation, 4 : Medium infestation, 5 : Heavy infestation.

Step 4. Infested plants (%)

The observation data may be used to determine the infested plants (%) in observation area. This infestation parameter is very useful, as many plants of one crop-germplasm might be infested by only a few insect-pests, or just a few plants of the another crop-germplasm might be attacked by large number of pests (81, 84).

Step 5. Population dynamics

Data from the observations in open field are also used to determine dynamics of insect-pest population on the assessed crop-germplasms. It may consider the pest population dynamics on certain germplasms during the growing season or the dynamics (population fluctuations) in the following seasons (9,64,84). The first insects appearance on the tested crop-germplasms, peak of pest density, and pest population break down as well as its disappearance from tested plants are determined (Fig. 4). The obtained data may be also analyzed for insect migration onto plants of the tested crop-germplasms (79).

Step 6. Population structure

When number of various morphs of the pest present on the tested crop-

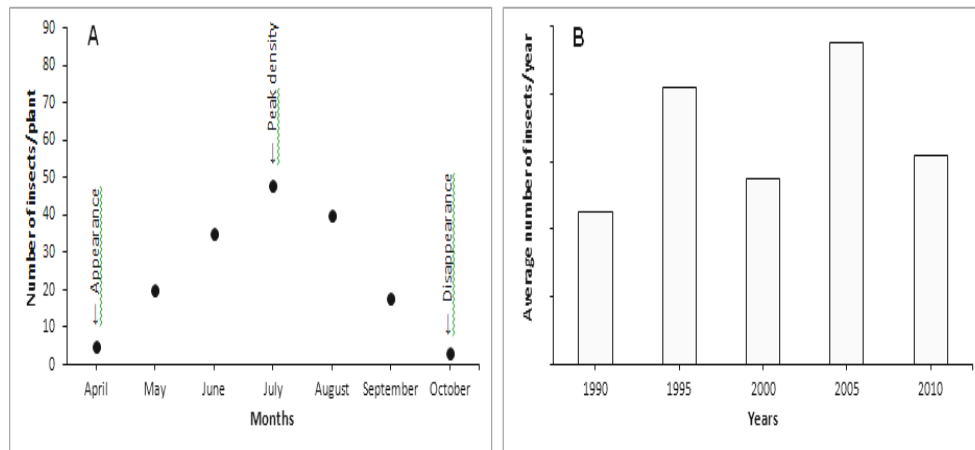


Figure 4. Scheme of the population dynamics. A: During the growing season; B: During 2- decades of field observations

germplasm is recorded, analysis of the population structure is possible. Generally, it helps to better understand growth and development of population (84). Usually, diversification into the following insect instars: *apterae* (adult wingless morphs), *alate* (adult winged morphs) and various stages of larvae is performed (Fig. 5).

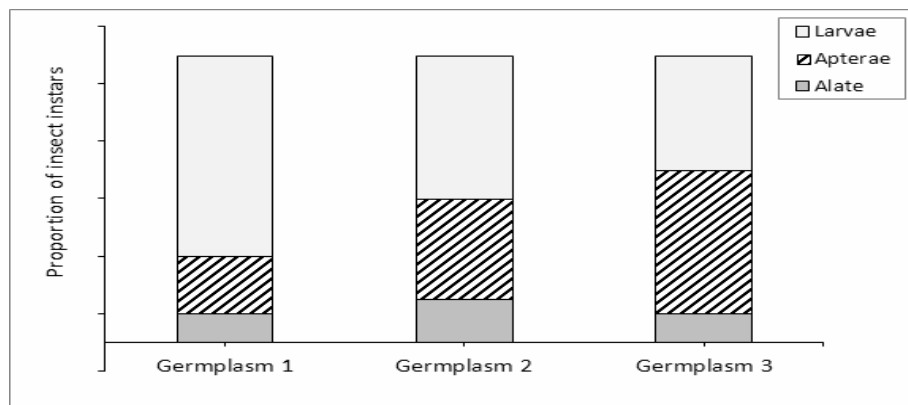


Figure 5. Comparison of pest population structure on plants of three crop-germplasms.

Step 7. Distribution on plants

Useful information about the pest performance on tested crop-germplasms might be obtained when its distribution on plant organs is recorded (Fig. 6). It is important in case of mowing insects, when longevity of insect population on vegetative and generative organs is analyzed and yield threshold is calculated.

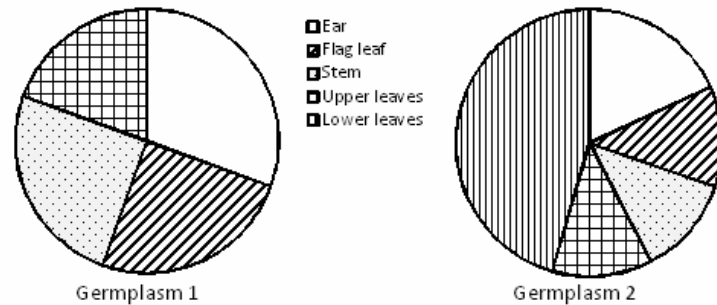


Figure 6. Comparison of distribution of pest morphs on two tested crop-germplasms.

8. Aggregation

Some insect-pests aggregate, while occurring on the infested plants (34,40), thus while the visits in the observation field, observer should make some notes describing pest aggregation on the tested plants. It might be important, since quite often in the places of aggregation the heaviest damages occur.

Step 9. Size of colony

Some insect-pests reproduce parthenogenetically and form colonies of different size on the infested plant surface. Well known group of such pests are aphids characterized by short generation time, viviparous reproduction, telescoping generations, and thelytokous parthenogenesis, what enables them to reach a high population densities in very short time (33). For such pests, the observer while monitoring the insect population in the field makes some additional notes about their distribution on the studied plant organs. The data should contain the informations about how many colonies occurred and their sizes.

Step 10. Clonal variability

The insect clonal variability can be extensive, since genetic recombination during the sexual phase of their life cycle allows them to produce various genotypes. For example, the aphids use the genotypes (clones, biotypes) for rapid spread in environment through the parthenogenetic phase, to reach a higher fitness (18,32). The major difference between the aphid clones are as follows: various body colours different ability to produce sexual morphs and various development and reproduction on resistant and susceptible crop germplasms (13,115).

Step 11. Duration of occurrence on plants

When the field observation is carried out through the whole vegetation season, it is easy to determine the duration of the insect-pests occurrence on the host-plants. Such information might be useful to calculate the crop threshold per infestation day or per pest number. The data might be valuable when the post-infestation effect on crop yield and its quality is determined.

Statistical analysis

When the observations are performed on several crop-germplasms, in at least three-four independent replicates, the analysis of variance (ANOVA), followed by Tukey's test is possible.

3.2. Egg laying behaviour

Many insect-pests lay eggs on the plant surface, which develops in the immature instars and then to adult morphs (13). Harris *et.al.* (55) and Harris and Foster (54) showed that ovipositioning females after landing on the host-plant always lay eggs.

Observation: Oviposition

Some phytophagous insect species become less selective about their host plants, when they have not recently oviposited and the egg load is large (10). Thus the egg count is very useful, to determine possible pest outbreaks and their preference to tested crop-germplasms in field.

Materials and equipment requirements

Seeds of test crop germplasms, observation field, sowing equipments, fertilizers, binocular, record cards, ball pen/pencil

Observation procedure

After the arrival of ovipositing females, the observer counts the number of eggs laid on randomly selected plants of test crop-germplasms. The counting procedures has to be adjusted to the required standard for certain crops and pests. Usually the eggs are counted on 25-100 plants randomly chosen across the field; 25-100 plants, chosen at random in rows of 20 to 30 m lengths inside the crop or 25-100 plants selected at random in serpentine route in studied field. Counts are made weakly or twice weakly during the crop-germplasms colonization period and the data are recorded in observation cards. The laying eggs behaviour should be monitored in at least 10-25 independent replicates.

Calculations

Obtained results are summarized and expressed as average number of eggs load per plant of test crop-germplasm, or total number of eggs load on all monitored plants of examined germplasm.

Statistical analysis

When least a few crop-germplasms are monitored and the proper replicates are performed, the analysis of variance, followed by Student *t*-test is recommended.

3.3. Occurrence on soil

When the insect-pests move on the plant surface or are attacked by predators they fall down on soil under the plants. Thus number of insects on the soil reflect their density on plants and may be used as indirect method to determine their numbers on monitored crop-germplasms (52,130).

Observation : Relation between insect density on plants and soil

In early season, a strong relationship occurs between the number of insects on the soil and on the control plants than the higher densities of insect later on the infested plant (27,61).

Materials and equipments required

Observation field, soil collection equipment, sodium chloride solution

Sampling procedure

Soil Samples upto 2-3 cm depth, from quadrates (20 cm x 20 cm) are taken from several areas of the observation field. The samples are stored at -20°C until sorted, and the insects are extracted from the soil by flotation in an agitated sodium chloride solution (132,133). These insects are counted and their density on plants of monitored crop-germplasms is projected.

Calculations

The indirect projection of pest density on the monitored crop-germplasms usually need to determine mode of relationships between insect number on plants and on the soil. Generally it is done in form of regression slope.

Statistical analysis

The obtained data are converted to the log of insect density on the soil (m^{-2}) and to the log of insect density on the plant surface (m^{-2}) and the slope of regression is calculated. The relationship is presented as regression slope and regression equation.

4. DETERMINING INSECT POPULATION IN FIELD: CAGE EXPERIMENTS

Detailed evaluation of insect population in field conditions is possible only when the plants are marked and isolated or caged (various type of cages and/or isolators). In the caged experiments, screening of crop-germplasms insect-pests gives precise data (43,44). Such experiments reject or minimize the effects of parasites, predators and weeds on studied crop-pest interactions. When the caged experiments are done in field an isolated control (without insects), prepared exactly like the group of infested plants is required.

4.1. Natural infestation

Some field experiments [randomly located plots with at least 3-4 replicates (81), fully randomized design are: randomized blocks, Latin square, split-plot designs (107,113) or transects (37)] are done at distances of 1m to 150m (Fig. 7). The experimental fields should be organized as per to the standard requirements of particular crop and the test germplasms should be sown, at least in 4 replicates each (pure-stock multiplication plots).

Experiment 1: Colonization in open field

After the insect-pests arrival, the field cages covered with nylon net with fine mesh are erected over the randomly selected plants and placed on experimental area (Fig. 8). The caged area should be adjusted to the test crops, *e.g.* for cereals 2m x 2m x 2m (149) or 3m x 3m x 2m (81) and should be comfortable to work in it. The cages should be

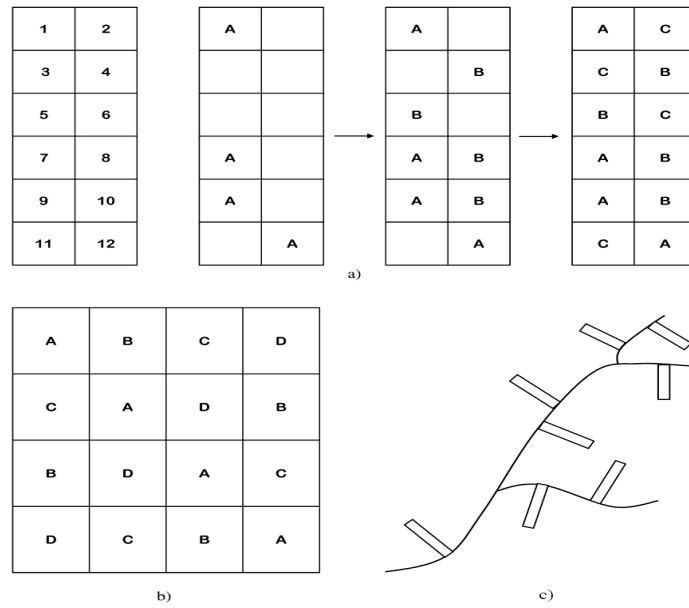


Figure 7. Various schemes of field experiments. a : Completely randomized blocks, b : Latin square, c : Transects

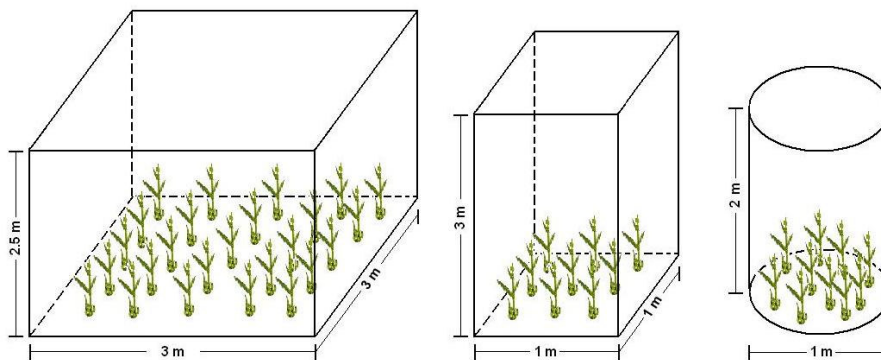


Figure 8. Types of Cover cages used in field experiments.

constructed of steel scaffolding and covered with nylon net (1 mm mesh). Depending on the experimental procedure, parasites, predators and weeds should be removed before the start of experiment.

Materials and equipment requirements

Experimental field, seeds of tested crop-germplasms, arriving insect-pest population, cover cages, plant markers, binocular, record cards, ball pen/pencil

Experimental setup

The cage monitoring should involve 25-100 plants/cage, chosen at random and marked and at least four independent replicates for each tested crop-germplasm needs to be performed. The counting is made weakly from the beginning of the experiment until the end of the pest occurrence on the host-plant. When the daily fecundity and/or longevity of reproduction is measured, daily inspections are required.

Calculations

Detailed measurements of pest abundance and its development on the tested crop-germplasms (density, infestation scales, aggregation, size of colony, population structure and dynamics, generation length, clonal variability; see section 3) might be calculated.

Statistical analysis

When few crop-germplasms are monitored and the proper number of replicates are used, the analysis of variance followed by Duncan's test is recommended.

4.2. Artificial infestation

Single plants of test crop-germplasms located under cover cages might be artificially infested with the certain level of individuals pests of the same developmental stage. Such plants are marked and clip-on cages (37,109,143), "Mylar" cylinders (59,69,148), nylon mesh sleeve cages (4,78) or different type of insulators are placed on the whole infested plants or selected organs (Fig. 9).



Figure 9. Examples of small cages used to isolate insects on single plants or their organs.

Experiment 1: Population development

Detailed monitoring of pest population development and its effect on the test crop-germplasms is followed till harvest. The experiment require at least 15-25 plants artificially infested in each cage with at least 4 replicates. In some experiments, an isolated control plants (without insect-pests) of the test crop-germplasms should be prepared.

Materials and equipment requirements

Experimental field, seeds of tested crop-germplasms, insect-pest population, cover cages, small cages or isolators, binocular, record cards, ball pen/pencil

Experimental setup

The artificially infested plants are monitored weekly, and on each occasion number of insect-pests per plant/organ is counted. At end of experiment, the cages are removed and infested and control plants of tested crop-germplasms are collected and further study of infestation effect may be conducted.

Calculations

Artificial infestation experiments allows the calculation of cumulative index of infestation, size of insect colony, population structure, population dynamics and duration of pest occurrence on test crop-germplasms (see procedures section 3). It also allows to determine precise effects of pest population level on infested plants of test crop-germplasms (see section 5.2).

4.3. Population parameters

When testing crop-germplasms in field to insect-pests, detailed population tests are required. Such experiments are done under cover cages on separately caged plants (clip-on cages), pest with individual pest on them.

Experiment 1: Demographic tests

These experiments allow precise record of demographic parameters such as : pre-reproductive period (larval development), daily and total fecundity, reproductive period, intrinsic rate of increase (r_m) and potential increase rate (PIR; e^{λ}), survival, post-reproductive period, and generation length in the field conditions (7,68).

Materials and equipment requirements

Experimental plots, seeds of test crop-germplasms, insect-pests, cover cages, clip-on cages or isolators, binocular, record cards, ball pen/pencil

Experimental setup

Single adult females are transferred onto single plants of test crop-germplasms, in 20-40 replicates and caged using: clip-on cages (93,149), small plastic cages (65), plastic PVC cylinders (77), muslin cages (93) or a cellar tube cages with the tops enclosed with mesh cloth (72,81). They are kept in cages until they start to reproduce. Then the adult and its progeny are discarded and one first instars nymph is retained in each cage and reared over its whole life span on plants of test crop-germplasm. While the experiment, the time from birth till the first reproduction, fecundity, reproductive rate per day, survival and the generation longevity are monitored everyday (81,93,117,151). All test insects should be from the respective stock cultures to avoid previous cultivar-rearing effect on the insect reproductive rates (2,81,89).

Calculations**Step 1. Pre-reproductive and post-reproductive period**

The obtained results are calculated in term of larval growth and development period [(i). Pre-reproductive period, (ii). Developmental period and (iii). Time from birth till first larviposition] and expressed in days (84,118). When the adult female stops to

reproduce, the post-reproductive period (time from the last birth till end of its life; in days) is determined.

Step 2. Reproduction

When the mature female starts to reproduce, the observer records number of its progeny born every day. After the end of experiment, total and average fecundity/day is calculated for each female, and the mean reproduction period and fecundity for the all monitored pest females on the tested crop-germplasms is determined (50,51,72).

Step 3. Life tables

Calculation of the reproductive rates requires preparation of life tables (21,28,99,132,141). They allow to determine the changes in survival of the observed females and in their fecundity during the experimental period. Below is example of such calculations for the experiment starting with 20 females (Table 1).

Table 1. An example of insect-pest life tables

Day	Number of females	Number of offsprings	l_x	m_x
1	20	0	1.0	0
2	20	0	1.0	0
3	20	0	1.0	0
4	18	0	0.9	0
5	18	0	0.9	0
6	18	0	0.9	0
7	17	10	0.85	0.56
8	17	31	0.85	1.82
9	14	45	0.7	3.21
10	14	53	0.7	3.79
11	10	35	0.5	3.50
12	9	30	0.45	3.33
13	6	18	0.33	3.00
14	5	12	0.25	2.40
15	2	3	0.10	1.50
16	1	2	0.05	2.00

Step 4. Intrinsic rate of natural increase

Plant-insect interactions are characterized by an intrinsic rate of increase (r_m), defined by Birch (12) as the constant "r" in the differential equation for insect population increase in an unlimited environment:

$$\sum \exp(-r_m x) l_x m_x = 1$$

Where: l_x : Changes of survival; m_x : Female fecundity; x . Appropriate short time unit of reproductive life

The equation combines several biological components and is very useful in biological and ecological understanding of insects. Wyatt and White (151) simplified the Birch's equation and developed new formula:

$$r_m = 0.738(\log_e M_d)/d$$

Where, d : Time taken (days) by nymph to reach reproductive maturity and produce the first nymph; M_d : Number of progeny produced in equal time from beginning of reproduction.

Another modification of r_m coefficient, uses the hours instead of days, to determine the intrinsic rate of natural increase (72).

Step 5. Potential increase rate

Potential increase rate (PIR) in each instar distribution, per block (cage) and treatment (crop-germplasm) on the sampling occasion can be calculated using the quick method of Hughes (63,64), or the maximum likelihood method of Forster (48).

Step 6. Longevity of reproduction

While characterizing the crop-germplasms influence on the insect-pests, the most important parameter is longevity of reproduction (reproductive period). This parameter suggests how suitable is the host-plant and to what extent it allows the female to reproduce (93). Thus the obtained data are analyzed in terms, when the reproduction starts and when is finished (in days from the beginning of experiment).

Step 7. Survival and mortality

Detailed cage experiments also allow determining the mean and total survival and mortality and expressing them as a percentage of monitored individuals for each test crop-germplasms (84). Quite often these parameters are expressed as mortality/survival rates (8,24).

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test is done to find the significant differences in the insect-pests performance on the test crop-germplasms.

5. FINDING CROP-GERMPLASMS RESISTANCE MECHANISMS AGAINST PESTS IN FIELDS

The field experiments also allow to screen level of resistance of test crop-germplasms to insect-pests, and help to determine the possible resistance mechanisms (92,110,111,125). The test crop-germplasms might be not colonized by the insect-pests (non-preference; antixenosis mechanism). Some of them are colonized but not allow the pest to perform well (antibiosis mechanism).

There are also crop-germplasms that are doing very well under the insects' attack or even overcame the injury effect on yield and its quality (tolerance mechanism). Antixenosis and antibiosis are resistance mechanisms that measure the effect of plant on the insect-pests' growth and development, instead the tolerance mechanism determines the pest effect on attacked plants.

5.1. Antixenosis

The host-plant selection by winged and/or wingless insects in the field mainly depend on food quality of crop-germplasms. Thus there is a group of choice tests that allow to describe how the insect-pests colonize the tested crop-germplasms (30). Quite often, at this early stage of colonization non-preference resistance mechanism occurs (110), which is also named antixenosis by Kogan and Ortman (76). Occurrence of antixenosis mechanism in field conditions is usually tested in a series of choice tests (131).

Experiment 1: Walk about test

One antixenosis screening technique is done under cover cages in the field is called primary “walk about test” (90). Selected plants of tested crop-germplasms (at least 15 plants for each stock) arranged randomly to the blocks are exposed to winged or wingless pests. The insect population is introduced as evenly as possible, by shaking infested plants over the tested crop-germplasms and the pests are left free to move and to increase their populations for 10 to 14 days.

Materials and equipment requirements

Experimental plots, seeds of tested crop-germplasms, insect-pest stock culture, cover cages, observation accessories

Experimental setup

After 1-2 weeks, the number of insects on plants of tested crop-germplasms is visually assessed and recorded. Video recording can also be done to observe the insects movement between the plants. If the population is pretty dense, it is better to take the plants to lab for detail inspection, after the end of experiment.

Calculations

The primary “walk-about test” determines how many insects chosen plants of the particular crop-germplasms under the cover cages in the field conditions. It is also possible to compare the level of infestation and percentage of infested plants of the test crop-germplasms. When the infestation is pretty high a 0-9 scoring scale might be applied; where 0 : No insects on plants, and 9 : Exceptionally heavy infestation (90). The mean of individual plant scores and the percentage of the resistant and susceptible crop-germplasms may be determined. When the scoring scales are used, separation of resistant (score ≤ 3) from susceptible (score ≥ 6) crop-germplasms is possible.

Statistical analysis

Analysis of variance (ANOVA), followed by Duncan’s test should be carried out to find the significant differences in selection of test crop-germplasms by the insect-pests.

Experiment 2: Test with equal level of infestation

In such test, the experiment is started with a defined uniform infestation *e.g.* 5 insects of same developmental stage per plants of test crop-germplasms (in 10-25 replicates). The insects are left free to move and to increase their populations on plants of test crop-germplasms for 10 to 14 days.

Materials and equipment requirements

Experimental plots, seeds of tested crop-germplasms, insect-pest stock culture, cover cages, observation accessories

Experimental procedure

Movement of insects on tested crop-germplasms is monitored in 2- weeks of experiments and on each occasion, number of insects of different developmental stages are recorded.

Calculations

The mean of the individual plant scores and the percentage of colonized plants of the crop-germplasms are determined.

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test is done to find the significant differences in the insect-pests choice between the tested crop-germplasms.

5.2. Antibiosis

A group of tests that allows to screen antibiosis of crop-germplasms to insect-pests is based on measurement of pest performance on plants of crop-germplasms in field conditions (11,25,127). Leather and Dixon (78) found, that the greater mean relative growth rate of the apterous females, the greater is their fecundity. Among methods determining antibiosis of test crop-germplasms to insect-pests in the field, the following tests may be done : reproductive growth rate and mean relative growth rate (weighting tests) (78), population tests (81) and EPG tests (85).

Experiment 1: Weighing tests of insects

The weighing tests show that the test crop-germplasms are suitable for the insect-pests as food source. It is known that the good host-plants offer the insects much better conditions for growth and development, an poor host-plants which don't fit well the pest feeding requirements (10,81,84,108).

Materials and equipment requirements

Experimental plots, field cages, clip-on cages, seeds of tested crop-germplasms, insect-pest stock culture, microbalance, record cards, ball pen/pencils

Experimental setup

Twenty apterous females are placed separately on twenty selected plants of test crop-germplasms in small clip-on cages or insulators. When the female give birth to first nymph, the female is discarded with other nymphs, instead the first nymph is weighted and placed back into the clip-on cage. After 4-days of development period (MRGR), or when it reaches maturity (RGR), is weighted again and its development on plants of particular crop-germplasm is determined

Calculations**Step. 1. Mean relative growth rate**

The mean relative growth rate (MRGR) is calculated using the formula given by Fisher (43) and further modified by Radford (116):

$$\text{MRGR} = (\ln W_{t_4} - \ln W_{t_1}) / (t_4 - t_1)$$

Where, W_{t_4} and W_{t_1} : Weights at fourth day and birth respectively, $(t_4 - t_1)$: Time taken to develop from birth to fourth day of insect development

Step. 2. Relative growth rate

Howard and Dixon (62) modified the MRGR formula for the aphids and named the relative growth rate (RGR; total developmental time):

$$\text{RGR} = \left(\frac{\ln W_t - \ln W_o}{t} \right)$$

Where, W_t and W_o : Weights at maturity and birth respectively, t : Time taken to develop from birth to maturity

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's test is done to find the significant differences in the pests performance on the tested crop-germplasms.

Experiment 2: Developmental tests

Plants of the tested crop-germplasms are artificially infested with pest individuals of same developmental stage and placed in clip-on small cages or insulators. Detailed observation of pest individuals development are recorded (20), on at least 20 plants of tested crop-germplasms and the following population parameters are calculated: pre-reproductive period, reproduction, daily fecundity, reproductive rate per day, intrinsic rate of natural increase (r_m), survival and the generation longevity (See methods described in section 4). Such experiments require at least 4 independent replicates for each crop-germplasm.

Experiment 3. Honeydew collection

Some laboratory screening tests might be successfully done in field conditions, on plants isolated under cover cages, when they are well organized. One of them is a honeydew collection test, perfectly showing the feeding activity of sucking-piercing pests on the tested crop-germplasms. The host-plants that better fit the insect-pests feeding requirements are usually preferred and sucking-piercing insects feeding on such plants produce bigger amounts of honeydew (114).

Materials and equipment requirements

Experimental plots, field cages, seeds of tested crop-germplasms, insect-pest stock culture, filter paper or TLC aluminium silica gel chromatography plate, impregnated

with ninhydrin (in butanol and acetic acid solution), or container with an oil, spectrophotometer, record cards, ball pen/pencil

Experimental setup

Individuals of sucking-piercing insects of same developmental stage are isolated on plants of test crop-germplasms and allowed to feed. Next, under the feeding side a piece of filter paper or TLC aluminium plate is placed (142) or small container with an oil (134). While the pest feeding the honeydew is produced and its droplets fall down on the filter paper, TLC plate impregnated with ninhydrin or into the oil container. After certain time, when the collection is stopped and the collected honeydew is analyzed, using quantitative and/or qualitative chemical methods. Some authors measured the frequency of honeydew production, using the 24h honeydew clock, and stain the amino acids occurred in the honeydew droplets (103,114,142).

Calculations

Amount of the honeydew produced and its composition is chemically determined in the laboratory and used as an antibiotic parameters of the tested crop-germplasms towards the insect-pests.

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test is carried out to find the significant differences in honeydew excretion while feeding on the tested crop-germplasms.

Experiment 4. EPG tests

One of the best method to screen crop-germplasms to sucking-piercing pests is EPG (Electric Penetration Graphs), invented by MacLean and Kinsey (95) and further developed as DC system by Tjallingii (139,140). This method allows comparing a probing and feeding activity of the sucking-piercing insects on the tested crop-germplasms, since insect feeding behavior is recorded as a specific EPG waveforms (Fig. 10). It is well known that on the resistant germplasms the pests do less probes into plant tissues and ingest far less phloem and xylem sap (19,85,112,123).

Materials and equipment requirements

Experimental plots, field cages, seeds of tested crop-germplasms, insect-pest stock culture, EPG system adjusted for the field study, PC analyzing EPG recordings)

Experimental setup

At least 20 individuals of sucking-piercing pests are connected to the EPG system and placed separately on plants of the tested crop-germplasms (one insect per plant; the same developmental stage) under the cover cages. The 4-8 h of the EPG continuous recordings are performed, using the computer operating EPG system. When the EPG recordings are done in the field conditions it is highly recommended to perform the tests at the same time, on the same organs and at the same growth stage of tested crop-germplasms. While the EPG recordings, duration and number of following insect activities

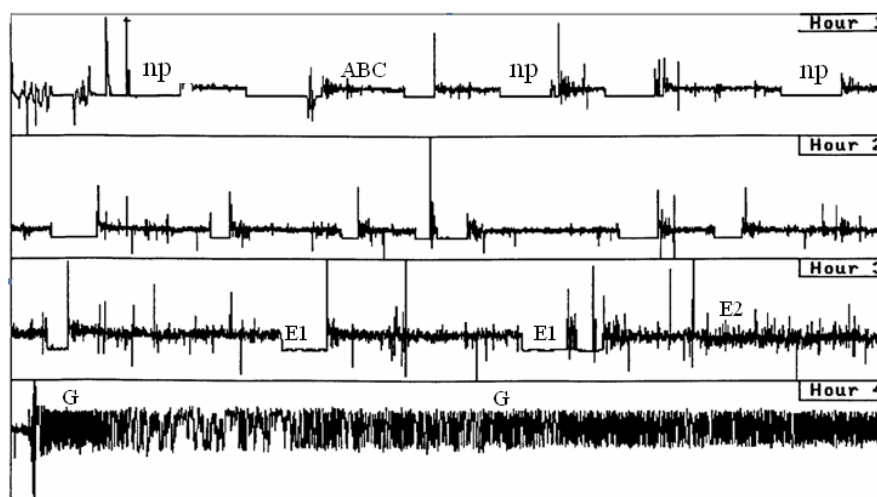


Figure 10. An example of the EPG graphs (86,139). N- non-probing behaviour; ABC: Penetration of peripheral plant issues (epidermis and mesophyll), F: Stylet activities within all plant tissues, E1: Salivation into the sieve elements, E2: Phloem sap ingestion and G: Xylem sap ingestion

is determined: non-probing, penetration of peripheral plant tissues (epidermis and mesophyll), stylet activities, salivation into the sieve elements, phloem sap ingestion and xylem sap ingestion (139,140).

Calculations

The obtained data are transformed and analyzed with the help of PC Stylet programme and number and duration of various feeding activities of sucking-piercing pests are evaluated (Table 2).

Table 2. Feeding activities calculated from the EPG recordings in field conditions (114)

Location of pest activity	Specification of the activity
Plant surface	Non-probing behaviour
	Start of probes into plant tissues
	Withdraw the stylets from plant tissues
	A waveform (stylet contacts on/off with epidermis cells)
Peripheral tissues (Epidermis/mesophyll)	B waveform (sheath salivation)
	C waveform (activities during stylet pathway)
	pd waveforms (intracellular punctures)
	E1 waveform (salivation into sieve elements)
Phloem phase (Sieve elements)	E1e waveform (puzzling E1 incident)
	E2 waveform (watery saliva secretion and passive ingestion of phloem sap from sieve elements)
Stylet movements (All tissues)	F waveform (mechanical stylet work)
Xylem phase (Xylem elements)	Gw waveform (active ingestion of xylem sap from xylem elements)
	Gp waveform (unknown xylem subactivity)

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's test is done to find the significant differences in the insect-pests performance on the test crop-germplasms. In addition, specific relationships between the measured parameters and level of test crop-germplasms resistance can be determined using the correlation coefficients and/or regression curves.

5.3. Tolerance

Tolerance resistance mechanism occurs when the production of plant biomass is carried out in spite of insect-pest attack (110). The tolerance tests allow to determine the insect injury and plant response to them, thus the less damage, the higher the level of the tolerance (119).

Experiment 1. Functional plant loss index

All the tolerance tests consists of two groups of plants of test crop-germplasms: control and artificially infested. Equal number of control and infested plants (25-100 plants; in 3-4 replicates) of same growth stage are prepared under field cover cages and separately caged using small cages or insulators. At particular growth stage, the group of infested plants is artificially infested with known level of insect-pests (5-15 individuals per plant), and allow to feed for certain period (2-4 weeks).

Materials and equipment requirements

Experimental plots, field cages, small cages or insulators, seeds of tested crop-germplasms, insect-pest stock culture, record cards, ball-pen/pencils

Experimental setup

At the end of feeding period, insect-pests are removed from plants and their population is counted. Then groups control and infested plants of tested crop-germplasms are carefully checked and damage caused by the pest is calculated.

Calculations

The functional plant loss (FPL) and functional plant loss index (FPLI) is calculated as per following formulae of Morgan *et.al.* (105):

$$FPLI = \left(1 - \left[\frac{Area_C - Area_T}{Area_C} \right] * [1 - Damage] \right) * 100$$

$$FPL = \left(\frac{Area_C - Area_T}{Area_C} \right) * 100$$

Where, $Area_C$: Leaf area of uninfested control plant; $Area_T$: Leaf area of infested plant; $Damage$ is the average damage rating based on predetermined numerical scale

In original Morgan (105) scale the rating was from 0 to 10, where 0 =No injury, instead 10= Dead leaf, but such scale quite often results in negative values of the parameters (119). Thus further development of formula resulted in a better understanding recovery aspects in tolerance mechanism.

Experiment 2. Weighting tests of plants

Major group of tolerance tests are done by plant weighting tests, where weight loss of infested plants of the test crop-germplasms is compared with weight of their control plants. Among such tests, the weight index, the tolerance index and the relative weight change are calculated.

Materials and equipment requirements

Experimental plots, cover cages, small cages, seeds of tested crop-germplasms, insect-pest stock culture, balance, record cards, ball pen/pencil

Experimental setup

Equal number of control and infested plants (of same size, similar biomass and growth stage) are selected for the experiment. Half plants are artificially infested by the same level of insects, isolated and allowed to feed for 2-weeks. Afterwards, the insect-pests are removed from the infested plants and the control and infested plants are weighted and the weighting indexes are calculated.

Calculations

Step 1. The weight index

The weight index (WI) developed by Bramel-Cox *et.al.* (16) allows to measure the difference in biomass produced by the infested and control plants of test crop-germplasms. The damage on a per single insect-pest basis is calculated as per the following formula of Bramel-Cox *et.al.* (16):

$$WI = \frac{W_C - W_T}{GB} \times 100$$

Where, W_C : Weight of control plants, W_T : Weight of the infested plants, GB : Number of insects on infested plants at the end of experiment

Step 2. Tolerance index

The tolerance index (TI) was proposed by Dixon *et.al.* (35). It takes into account crop-germplasm to crop-germplasm difference by dividing the insect-caused loss in biomass ($W_C - W_T$) of the tested plants by weight of the control plants or the attacked plant organs to get the proportional weight loss.

The tolerance index (TI) is calculated as under :

$$TI = \frac{\frac{W_C - W_T}{W_C}}{GB} \times 100$$

Where, W_C : Weight of control plants, W_T : Weight of infested plants, GB : Number of insects on the infested plants at the end of experiment

The above formula may be also used as Index B after Robinson *et.al.* (122), where the insect weight is changed into the insect numbers.

Step 3. Relative weight change

The tolerance test so-called a relative or proportional weight change (DWT) was proposed by Reese *et.al.* (119). The DWT test represents a good method to know the treatment response, especially after it is determined that insect number sometimes don't have an effect on plant damage. This test also allows to eliminate the combined effects of antibiosis and tolerance mechanisms. The relative weight change is calculated as under:

$$DWT = \frac{W_C - W_T}{W_C} \times 100$$

Where, W_C : Weight of control plants, W_T : Weight of infested plants at the end of experiment

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test is done to find the significant effects of insect-pests on the produced biomass by the tested crop-germplasms. In addition, specific relationships between the measured parameters and level of tested crop-germplasms resistance might be determined using the correlation coefficients and/or regression curves.

Experiment 3: Loss of chlorophyll

Chlorophylls a and b are universal green pigments that are essential catalysts of photosynthesis. Some earlier study showed a significant decomposition of chlorophylls within the pest attacked plant tissues (46,47). It is important, since any damage of the chlorophylls result in decreasing the biomass production of higher plants. Thus assessment of chlorophylls in infested tissues is very good tool to determine the crop-germplasms tolerance insect-pests.

Materials and equipment requirements

Experimental plots, cover cages, small cages, seeds of tested crop-germplasms, insect-pest stock culture, chlorophyll meter, analytical balance, chemicals, spectrophotometer

Experimental setup

Control and infested plants (equal number, the same size, and growth stage) are artificially infested by the same level of insects, isolated and allow the pests to feed on them for 2-weeks. After that period the insect-pests are removed from the infested plants. The chlorophyll content within the control and the infested leaves is measured using the Chlorophyll meter SPAD-502 and/or acetone extracts of plant tissues.

Step 1. Within plant tissues

Chlorophyll meter (SPAD-502, Konica Minolta Photo Imaging Inc.) allows to measure the chlorophyll content of leaves without causing damage to plants. The measurements are taken by simply inserting the infested and control leaves and closing the measuring head. Results of measurements are expressed in SPAD values. A decrease in the SPAD value of infested leaves in comparison with SPAD value of control leaves, indicates a decrease in chlorophyll content. While it is comparing the tested crop-germplasms, it is possible to determine the presence of tolerance resistance mechanism.

Step 2. Within plant extracts

1g of control and infested leaves of tested crop-germplasms is homogenized with 5 mL of 50 mM potassium phosphate buffer (pH 6.5), containing 350 mM sucrose to obtain the chloroplasts' suspension. The homogenate is filtered through double layer of cheesecloth and centrifuged at 200 x g for 10 min at 4 °C. The supernatant is discarded and the pellet containing the unbroken cells is further centrifuged at 1,000 x g for 10 min at 4 °C. The pellet is gently dissolved in the chloroplasts isolation solution. Next, the supernatant is removed and the chloroplasts' suspension is transferred in to 15 ml of 80% of acetone, mixed and centrifuged at 1,000 x g for 5 min. The total chlorophyll content in the supernatant is measured at 652 nm and calculated according to equation given by Arnon (3) formula:

$$C = \frac{A}{El} = \frac{A_{652}}{34.5 \times l}$$

Where, C : Concentration of chlorophyll in mg/mL, A_{652} : Absorption of chlorophyll at 652nm, E : Absorption coefficient for chlorophyll, 34.5 mL/(mg x cm), l : Path length of cuvette

When the chlorophyll a and b content is determined separately, the absorbance of acetone extracts at 645nm and 663nm is measured respectively, and the chlorophylls concentrations (mg/g) are calculated as per Holden (60):

$$\text{Chlorophyll a} = 12,21 \times A_{663} - 2,81 \times A_{645}$$

$$\text{Chlorophyll b} = 20,13 \times A_{645} - 5,03 \times A_{663}$$

6. CONCLUSIONS

There are many advantages of doing field experiments, there are also good reasons to attempt to do at least part of the screening crop-germplasms towards insect-pests through laboratory investigations. The principal problem in the field tests is the unpredictability of weather conditions, since on cool and rainy days the insects' activity is pretty low. Another problem might be very low pest density in the field, what may unable to get the precise data on the studied insect-plant interactions. Thus the experimenter should be realistic to perform the screening of crop-germplasms towards insect-pests in

the field conditions. When they are difficult it might be better to collect the insect-pests in the field and do at least part of the screening in the laboratory conditions.

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