

ELECTROPHORETIC ANALYSIS OF 12 PROTEINS IN NATURAL POPULATIONS OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE)*

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ABSTRACT

Spodoptera frugiperda (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is considered to be one of the most serious pests for crops such as corn, rice and other Gramineae, and its control is of great economic importance.

Studies on this species have mainly focussed on the agricultural damage caused by the pest and on its control. There are practically no studies on genetic variability at the protein level within and among *S. frugiperda* populations.

The objective of the present study was to determine the electrophoretic patterns of twelve proteins (peptidases, carboxylesterases, malic enzyme, glucose dehydrogenase, alcohol dehydrogenase, glycerol-3-phosphate dehydrogenase, isocitrate dehydrogenase, leucine-aminopeptidase, nonspecific proteins, phosphoglucomutase, malate dehydrogenase, and superoxide dismutase) in natural *S. frugiperda* populations, as well as the number of loci involved, and to identify possible allele variants.

Six of the twelve proteins studied (malic enzyme, glucose dehydrogenase, alcohol dehydrogenase, peptidase, superoxide dismutase, and malate dehydrogenase) are described for the first time for this species. Twenty-two loci were detected, 13 of which presented genetically determined variation. Statistical analysis of the population data is in preparation.

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INTRODUCTION

Spodoptera frugiperda (J.E. Smith, 1797) is an insect of the order Lepidoptera, family Noctuidae, and is considered to be a New World species (Todd and Poole, 1980). It is an agricultural pest which, according to Luginbill (1928), originates in tropical and subtropical regions of the Western Hemisphere and is distributed from Southern Canada to Chile and Argentina (Todd and Poole, 1980). In the Americas, *S. frugiperda* is considered to be one of the major pests of corn, rice, sorghum and forage grasses (Wiseman *et al.*, 1966; Carvalho, 1970; Sparks, 1979; Pantoja *et al.*, 1986), which are basic foodstuffs for many human populations and which are also used for the preparation of animal rations.

In general, few agricultural pests have been characterized electrophoretically for genetically determined differences among populations, and *S. frugiperda* is not an exception to this rule. Studies on the protein variability of this species have been conducted by Pashley *et al.* (1985) on populations from Mexico, the United States and Porto Rico in order to establish the migratory routes of these insects among these countries.

In the present study we report the electrophoretic patterns of twelve proteins analyzed in pupae from two natural populations of *S. frugiperda*. The number of active zones and of loci involved were determined and possible allelic variants were identified. Statistical analysis of the population data is currently in preparation.

MATERIAL AND METHODS

Samples of natural *S. frugiperda* populations were collected from cornfields at the Experimental Agricultural Station of Sertãozinho, Department of Agriculture of the State of São Paulo (SP), from November to December 1984, and at a rural site in the municipality of Londrina, State of Paraná (PR) in February, 1985. Insects in the final larval stages were collected and maintained in the laboratory until the pupal phase, when they were stored frozen at -20°C until the time for use.

The pupal stage was ideal for analysis because there was no problem of extract contamination with food, it was possible to identify the sex of each individual, and a larger number of activity zones could be observed, with better visualization in most cases.

Individual samples were homogenized in 0.5% 2-beta-mercaptoethanol (v/v) and centrifuged for 20 minutes at 3000 rpm at room temperature. Whatmann No. 3 filter paper was used to absorb the supernatant for analysis by horizontal gel electrophoresis. Two types of gel were used, i.e., 10.5% starch (Smithies, 1955) and starch-agarose at respective concentrations of 2% and 0.8%. Buffer solutions and

reaction mixtures were prepared by the methods of Harris and Hopkinson (1976) and Lima (1989).

Table I summarizes the electrophoretic conditions used for the study of the following proteins: peptidases (PEP), carboxylesterases (EST), malic enzyme (ME), glucose dehydrogenase (GDH), alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), glycerol-3-phosphate dehydrogenase (G-3-PDH), leucine-aminopeptidase (LAP), nonspecific proteins (PT), phosphoglucomutase (PGM), superoxide dismutase (SOD), and malate dehydrogenase (MDH).

Table I - Electrophoretic conditions utilized for the study of different proteins in *S. frugiperda*.

Protein	Buffers		Gel type	Migration time (hours)	V/cm
	Bridge	Gel			
PEP, EST	0.3 M Borate pH 8.0	0.076-0.007 M Tris-Citrate pH 8.5-8.6	starch	5.5	6
ME, GDH, ADH	0.3 M Tris-HCl pH 8.6	0.02 M Tris-HCl pH 8.6	starch	16	2.5
IDH, G-3-PDH	0.240-0.15 M Phosphate- Citrate pH 8.6	1:40 dilution (v/v) of the bridge buffer	starch	17	3
LAP, PT	0.3 M Borate pH 8.0	0.017-0.0023 M Tris-Citrate pH 8.0	starch	5.5	2
PGM, SOD, MDH	0.22 M TEMM pH 7.4	1:15 dilution (v/v) of the bridge buffer	starch- agarose	5	6

RESULTS AND DISCUSSION

When interpreting the results we numbered the zones of activity according to their electrophoretic mobility from the anode toward the cathode. The nomenclature for the designation of loci and alleles was the same as that proposed by Shows *et al.* (1979) for human genes.

The electrophoretic pattern of each of the 12 proteins studied, the number of loci involved and their possible allelic variants are given below.

Peptidases

The electrophoretic pattern of *S. frugiperda* peptidases is formed by two anodic activity zones designated according to the migration of PEP1 and PEP2, which can be visualized throughout the ontogenetic development.

The two zones were variable in the populations analyzed. The electrophoretic phenotypes observed suggest that PEP1 is genetically controlled by one locus with three codominant alleles, *PEP1*1*, *PEP1*2* and *PEP1*3*, and that PEP2 is controlled by a locus with two, also codominant, alleles, *PEP2*1* and *PEP2*2*. Heterozygous individuals presented three bands for PEP1 and two for PEP2. Figure 1 illustrates the different electrophoretic phenotypes detected for this protein.

Pashley *et al.* (1985), who also studied the peptidases of *S. frugiperda*, detected two zones, each coded by one locus: *PEPF* with five alleles, two of them extremely rare, and *PEPS* with three alleles, one of which appeared only in one of the eleven populations investigated.

Among lepidopterans such as *Lymantria dispar* (Lymantriidae) (Harrison *et al.*, 1983) and *Hemileuca olivae* (Saturniidae) (Dubach *et al.*, 1988), only one PEP locus was detected, which was variable in both cases. In *Anticarsia gemmatalis* (Noctuidae), Pashley and Johnson (1986) detected two variable loci.

Carboxylesterases

Under the conditions used, the electrophoretic pattern of *S. frugiperda* esterases was found to consist of three activity zones. A fourth zone composed of two weakly staining bands and not always present in pupae was visualized between EST2 and EST3.

The more anodic esterase EST1 was detected only by specifically using 4-methyl umbelliferone ester, whereas EST2 and EST3, in addition to being detected with the use of this substrate, were also detected more appropriately with the use of

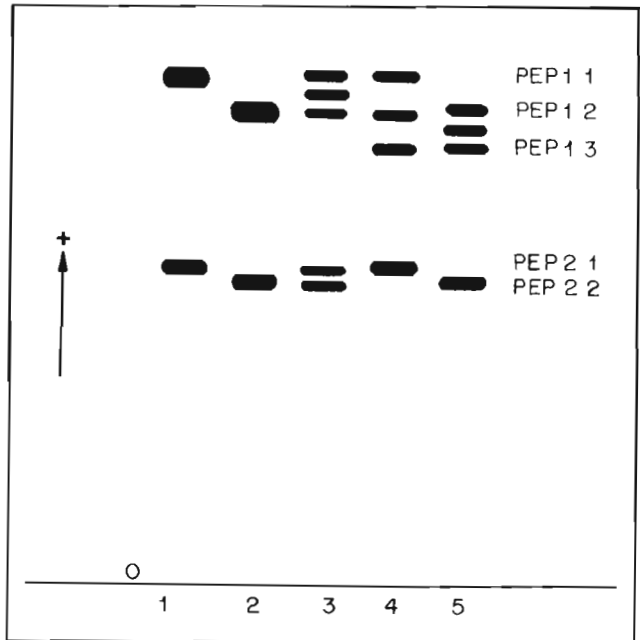


Figure 1 - Schematic presentation of the different electrophoretic phenotypes detected for peptidase in extracts of *S. frugiperda* pupae. For PEP1, the following phenotypes were obtained for each sample: PEP1 1 (sample 1), PEP1 2 (sample 2), PEP1 1-2 (sample 3), PEP1 1-3 (sample 4) and PEP1 2-3 (sample 5). For PEP2: PEP2 1 (samples 1 and 4), PEP2 2 (samples 2 and 5), and PEP2 1-2 (sample 3).

naphthyl esters. The three zones were present in all phases of ontogenetic development.

In the analyses performed on the two populations, EST1 and EST3 presented phenotypic variants, suggesting that the *EST1* locus may have two codominant alleles, *EST1*1* and *EST1*2*, and that the *EST3* locus may have at least 6 codominant alleles, i.e., *EST3*1*, *EST3*2*, *EST3*3*, *EST3*4*, *EST3*5* and *EST3*6*. The heterozygous individuals presented 3 bands for EST1 and/or EST3. The phenotypes detected for the carboxylesterases are shown in Figure 2.

In all analyses performed, EST2 consisted of three activity bands (Figure 2) of very close migration and of identical staining intensity. We believe that this region may be coded by a single locus but we do not rule out the possibility that each of these isozymes may be coded by different loci. We have no conclusive data to opt for one of these two possibilities.

Pashley *et al.* (1985) detected six alleles for the EST3 region, but did not comment on the number of loci or alleles involved with respect to EST1 and EST2.

In a study of adults from natural populations of *S. exempta*, Den Boer (1978) found only one zone of esterase activity for which he postulated the existence of a locus with three alleles.

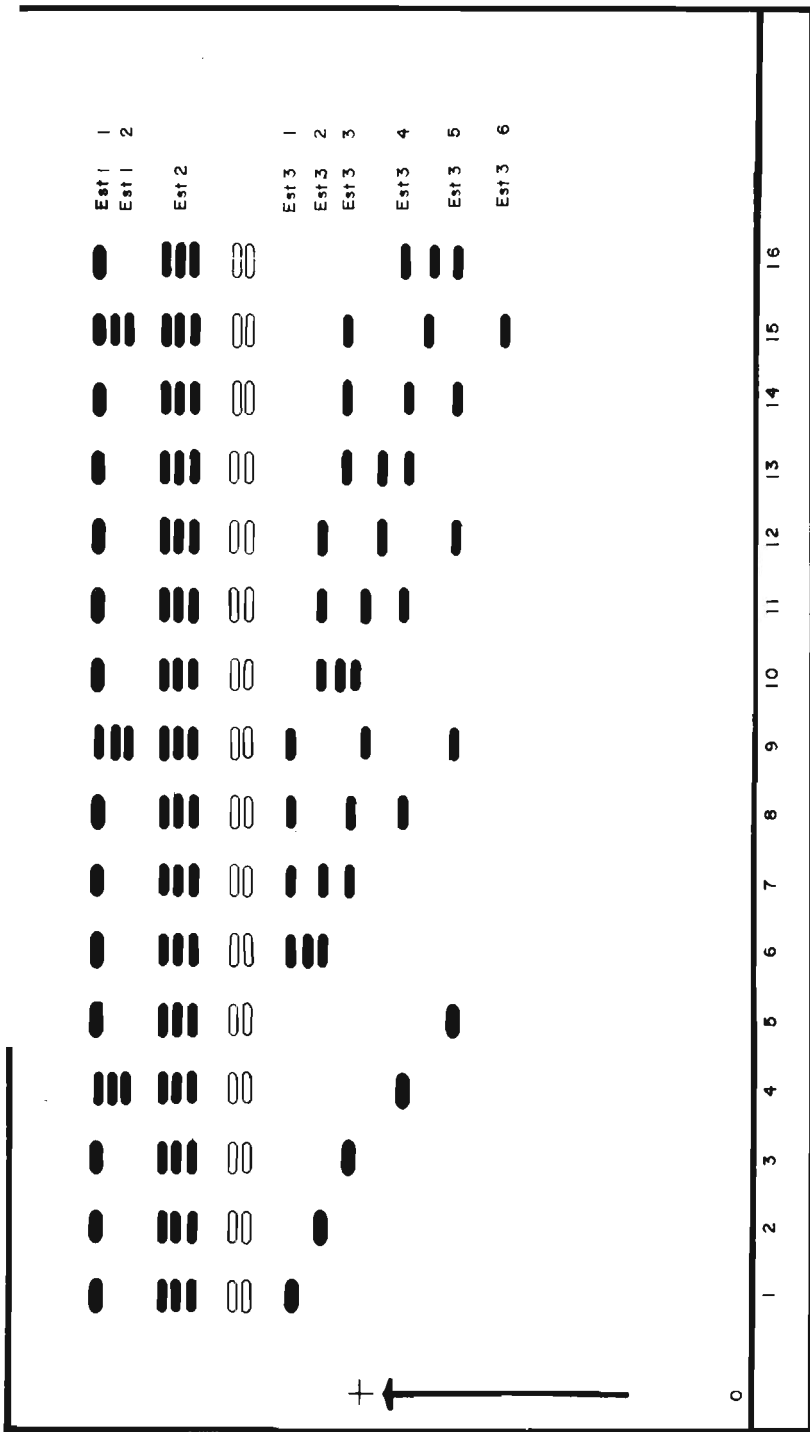


Figure 2 - Schematic presentation of the different electrophoretic phenotypes detected for carboxylesterases in extracts of *S. frugiperda* pupae. Sample 1, EST3 1; sample 2, EST3 2; sample 3, EST3 3; sample 4, EST3 4; sample 5, EST3 5; sample 6, EST3 1-2; sample 7, EST3 1-3; sample 8, EST3 1-4; sample 9, EST3 1-5; sample 10, EST3 2-3; sample 11, EST3 2-4; sample 12, EST3 2-5; sample 13, EST3 3-4; sample 14, EST3 3-5; sample 15, EST3 3-6; sample 16, EST3 4-5. Phenotypes EST1 1-2 (samples 4, 9 and 15), and the remaining ones, EST1 1.

Carboxylesterases have been extensively investigated in different lepidopteran families, always presenting several activity zones, at least one of which is variable (Lokki *et al.*, 1975; Sluss *et al.*, 1978; Stock and Castrovillo, 1981; Harrison *et al.*, 1983; Whillhite and Stock, 1983; Pashley and Johnson, 1986; Menken, 1987).

Malic enzyme

In *S. frugiperda*, malic enzyme presented only one activity zone which showed anodal migration and was monomorphic (Figure 3B). This zone is present in all phases of ontogenetic development and progressively becomes more deeply stained from the prepupal to the adult phase.

Studies by Den Boer (1978) have shown that in *S. exempta* malic enzyme is coded by a locus with two alleles. The same result has been observed for other lepidopterans. In some species malic enzyme is monomorphic including: *Lymanthria dispar* (Harrison *et al.*, 1983), 25 species of the Tortricidae (Pashley, 1983), and in *Anticarsia gemmatalis* (Pashley and Johnson, 1986). In others it is variable: *Solenobia triquetrella* (Psychidae) (Lokki *et al.*, 1975); several species of the genus *Speyeria* (Nymphalidae) (Brittnacher *et al.*, 1977); in two species of the genus *Heliothis*, family Noctuidae (Sluss *et al.*, 1978), and in five species of the genus *Choristoneura*, family Tortricidae (Stock and Castrovillo, 1981; Willhite and Stock, 1983).

Glucose dehydrogenase

Under the electrophoretic conditions used, we considered only one zone of glucose dehydrogenase activity, GDH1, which is more anodal and is present in all phases of ontogenetic development; the other GDH isozymes could not be properly characterized.

Analysis of pupal extracts in both populations revealed the presence of three electrophoretic phenotypes (Figure 3A) for GDH1, which suggest that this enzyme may be controlled by one locus, *GDH1*, with two codominant alleles, *GDH*1* and *GDH*2*.

Generally speaking, GDH is not an enzyme studied in lepidopterans.

Alcohol dehydrogenase

In *S. frugiperda*, the electrophoretic pattern of alcohol dehydrogenase is formed by three cathodal activity zones denoted ADH1, ADH2 and ADH3, according to their migration.

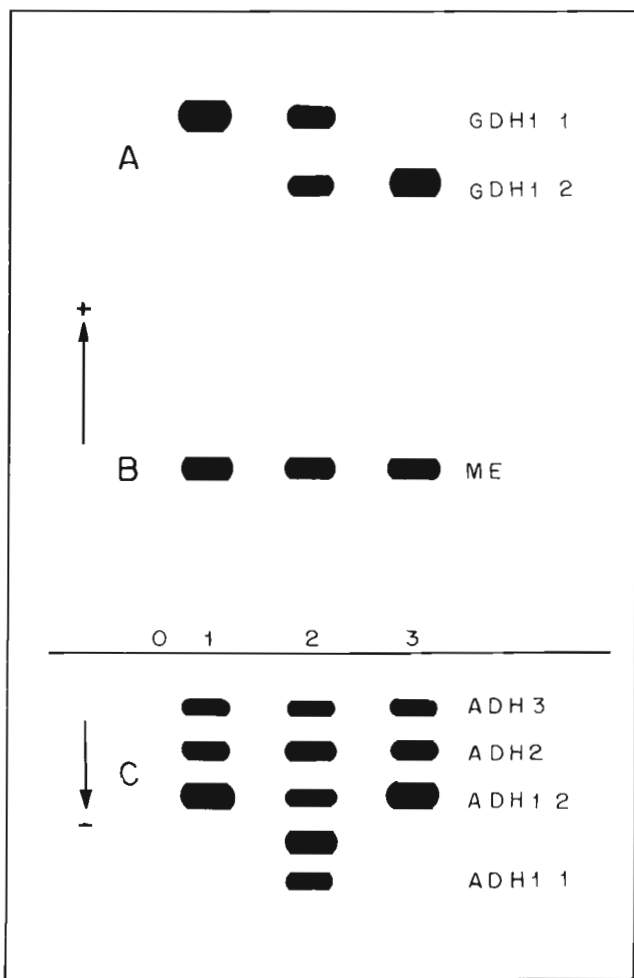


Figure 3 - Schematic presentation of the electrophoretic patterns obtained for extracts of *S. frugiperda* pupae. (A) Phenotypes detected for glucose dehydrogenase. Sample 1, GDH1 1; sample 2, GDH1 1-2; sample 3, GDH1 2. (B) Malic enzyme. (C) Phenotypes detected for alcohol dehydrogenase. Samples 1 and 3, ADH1 2; sample 2, ADH1 1-2.

This pattern varies according to the phase of ontogenetic development. From egg to 3rd-instar larvae the three isozymes are not detected; 4th- and 5th-instar larvae present ADH1 with reasonable staining intensity; 6th- and 7th-instar larvae, in addition to deeply-staining ADH1, present ADH2 with moderate staining; prepupae present all three isozymes, with ADH3 showing very weak staining at the beginning of this phase and becoming more deeply stained as the phase progresses. The pupal phase up to the 5th and 6th day is characterized by the three enzymes, all of them deeply stained, and ADH1 showing the highest staining intensity; as pupal age

progresses, ADH3 and ADH2 start to decrease in intensity. Adults only present ADH1, with weak staining intensity.

Even though the phenotypes detected for ADH could be interpreted in several manners, we consider the three isozymes to be the products of three distinct loci, ADH1, ADH2 and ADH3, which may be activated at different phases of ontogenetic development. The phenotypic variants detected for ADH1 may be determined by two codominant alleles at the ADH1 locus, ADH1*1 and ADH1*2 (Figure 3C).

Isocitrate dehydrogenase

Analysis of pupal extracts of *S. frugiperda* revealed an electrophoretic pattern consisting of two bands of isocitrate dehydrogenase activity, both of them of anodal migration, which were designated IDH1 and IDH2. The electrophoretic pattern of IDH varied according to ontogenetic development. In addition to the IDH1 isozyme which was visualized from the larval to the adult stage, two more anodal isozymes could be identified depending on the life cycle phase. Further studies are needed before more definite conclusions can be reached about these isozymes. IDH2 was detected from the prepupal to the adult stage.

Phenotypic variants for IDH1 and IDH2 were detected in the population analyses, suggesting the existence of an *IDH1* locus with two codominant alleles, *IDH1*1* and *IDH1*2*, for the Sertãozinho population, and a third allele, *IDH1*3*, in addition to the first two for the Londrina population (Figure 4A). The data also suggest that IDH2 may be coded by the *IDH2* locus in both populations and that this locus may have three codominant alleles, *IDH2*1*, *IDH2*2* and *IDH2*3*. Figure 4A presents two phenotypes detected for this zone. Heterozygous individuals for both IDH1 and IDH2 presented three bands.

In the *S. frugiperda* populations analyzed by Pashley *et al.* (1985), two IDH loci (*IDHS* and *IDHF*), possibly having three alleles each were detected. Den Boer (1978) observed two activity zones for IDH in *S. exempta* but did not make any genetic comments.

Isocitrate dehydrogenase has been investigated in other lepidopterans in which, as observed in *S. frugiperda*, it presented two activity zones determined by two loci which usually have more than one allele, as was the case for *Solenobia triquetrella* (Lokki *et al.*, 1975), *Lymantria dispar* (Harrison *et al.*, 1983), 25 species of the Tortricidae (Pashley, 1983), *Anticarsia gemmatalis* (Pashley and Johnson, 1986), and *Euphydryas* spp. (Nymphalidae) (Brussard, 1989). A single locus with genetic variation has been determined in *Choristoneura retiniana* and *C. fumiferana* (Stock and

Castroville, 1981), in several species of the genus *Speyeria* (Brittnacher *et al.*, 1977), and in *Heliothis virescens* and *H. zea* (Sluss *et al.*, 1978).

Glycerol-3-phosphate dehydrogenase

The electrophoretic pattern of glycerol-3-phosphate dehydrogenase (G-3-PDH) detected in pupal extracts of *S. frugiperda* consisted of a single activity band of anodal migration. We consider this enzyme to be coded by the *G-3-PDH* locus which may be monomorphic (Figure 4B). G-3-PDH is first detected during the last larval stages (6th and 7th) and the staining intensity of the band increases with pupal and adult age.

In the studies by Pashley *et al.* (1985), G-3-PDH monorphism was observed. In *S. exempta*, Den Boer (1978) identified one G-3-PDH activity band which may be coded by a locus with two alleles. Studies conducted on other lepidopterans have also detected a single G-3-PDH band coded by a single locus. In some species, G-3-PDH was found to be monomorphic: *Solenobia triquetrella* (Lokki *et al.*, 1975), *Lymantria dispar* (Harrison *et al.*, 1983), and 21 species of the family Tortricidae (Pashley, 1983). In others, G-3-PDH is polymorphic: three species of the genus *Speyeria* (Brittnacher *et al.*, 1977), *Heliothis virescens* and *H. zea* (Sluss *et al.*, 1978), *Choristoneura occidentalis* and *Archips argyrosipilus* (Pashley, 1983), and *Euphydryas* spp (Brussard, 1989).

Leucine-aminopeptidase

The electrophoretic pattern of leucine-aminopeptidase detected here consisted of a main zone of anodal activity present throughout the life cycle of *S. frugiperda* but staining more intensely after the 7th larval instar.

A second, less anodic activity zone was also detected but the electrophoretic conditions used were not ideal for its characterization.

In the Sertãozinho populations, LAP was monomorphic, and in the Londrina population it showed phenotypic variants whose electrophoretic pattern consisted of two activity bands. These phenotypes suggest that in this population the *LAP* locus may consist of two codominant alleles, *LAP*1* and *LAP*2* (Figure 5A).

In a study of 11 *S. frugiperda* populations, Pashley *et al.* (1985) described the *LAPS* locus, which may be variable in seven of them and may have two alleles. For *Heliothis virescens* and *H. zea* (Sluss *et al.*, 1978), three LAP loci were detected, all of them variable. Three loci were reported for five *Choristoneura* species (Stock and Castroville, 1981), one of them variable in all of these species and the other monomorphic in *C. occidentalis*; five variable loci for *Anticarsia gemmatalis* (Pashley and

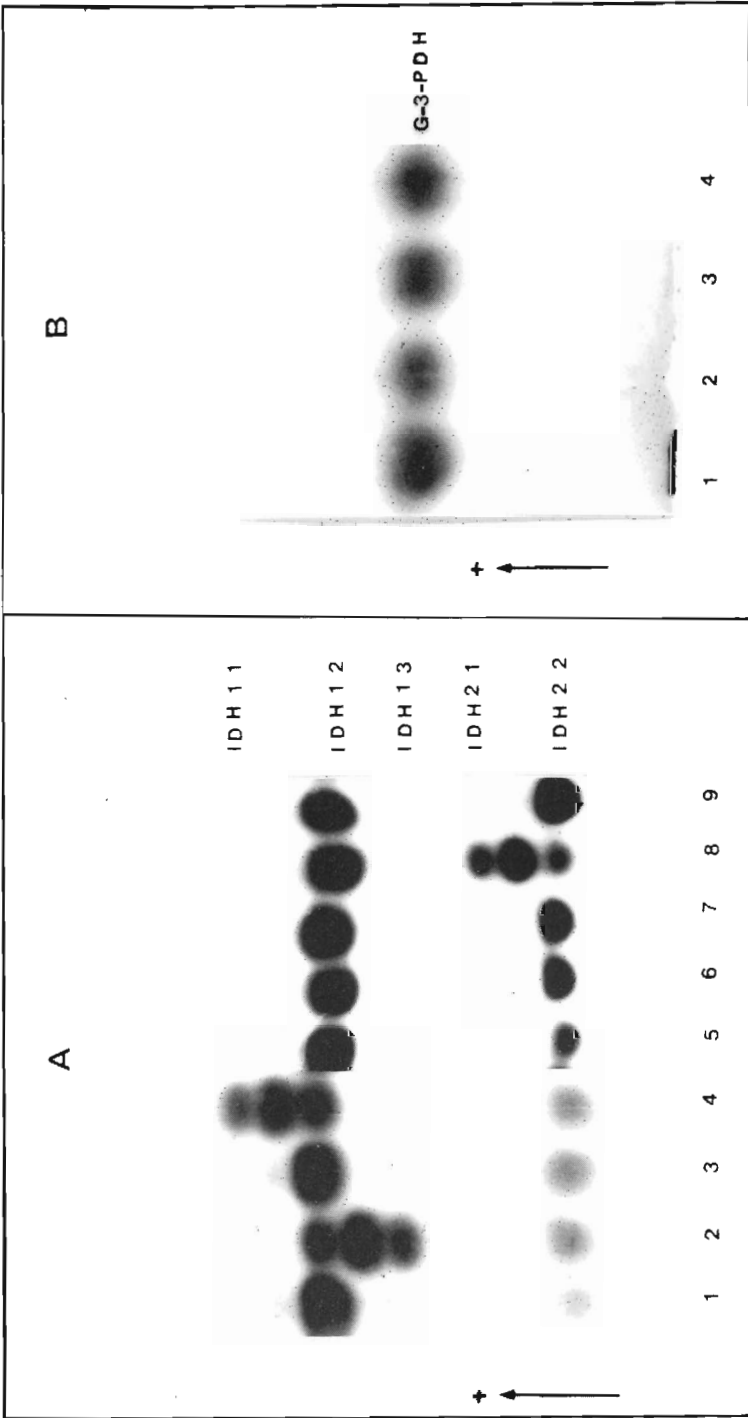


Figure 4 - Photographs of the electrophoretic patterns for extracts of *S. frugiperda* pupae. (A) Isocitrate dehydrogenase. Sample 2, IDH1 2-3; sample 4, IDH1 1-2; remaining samples IDH1 2; sample 8, IDH2 1-2; remaining samples IDH2 2. (B) Glycerol-3-phosphate dehydrogenase.

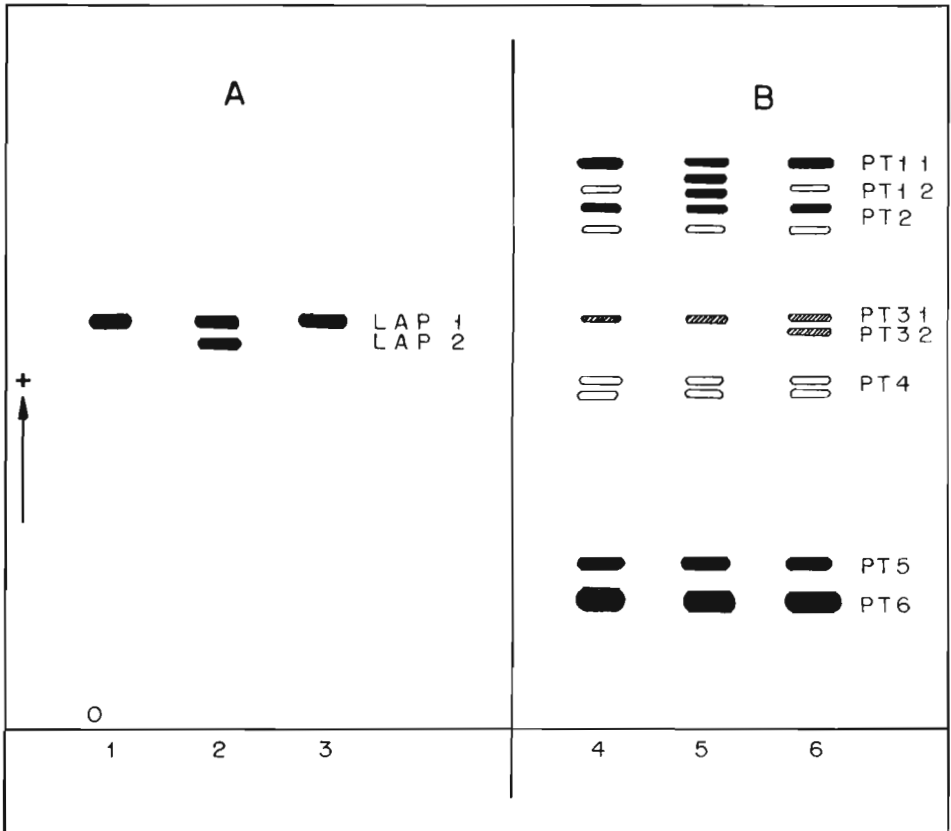


Figure 5 - Schematic presentation of the electrophoretic phenotypes detected in extracts of *S. frugiperda* pupae. (A) Leucine-aminopeptidase. Samples 1 and 3, LAP 1; sample 2, LAP 1-2. (B) Nonspecific proteins. Sample 4, PT1 1 and PT3 1; sample 5, PT1 1-2 and PT3 1; sample 6, PT1 1 and PT3 1-2.

Johnson, 1986), and one locus in 19 species of the Tortricidae, which was variable in four of them (Pashley, 1983).

Nonspecific proteins

The nonspecific proteins of *S. frugiperda* visualized with Amido-Black 10B showed an electrophoretic pattern consisting of six bands. The most anodal protein, PT1, was detected from the 6th larval instar to the end of the pupal stage. The phenotypic variants detected suggest that the *PT1* locus may have two codominant alleles, *PT1*1* and *PT1*2*, with heterozygous individuals showing three bands.

Between PT1 and PT2 there was a much weaker-staining band which, however, was not present in all electrophoretic analyses and had the same migration as the least anodic band of the PT1 1-2 heterozygote. PT2 can be visualized from the 3rd larval instar to the 10-day old adult phase. There was a weakly-staining band with slightly less anodal migration than PT2 which was not always observed in the electrophoretic analyses.

Present only in the pupal phase, PT3 is formed by a not very deeply staining band. Phenotypic variants were detected for PT3, suggesting that the *PT3* locus, which codes for this protein, may have two codominant alleles, *PT3*1* and *PT3*2*. Heterozygous individuals presented two bands.

PT4 consists of two weakly staining bands that are not always detected in all analyses and is only observed in the pupal phase. PT5 and PT6 are present from the 3rd larval instar to the final pupal stage and are deeply staining, though staining becomes weaker during the adult phase. We consider each of these three bands to be coded by one locus which is monomorphic for the populations analyzed.

Figure 5B schematically illustrates the electrophoretic pattern of nonspecific proteins and the different phenotypes detected for PT1 and PT3.

Phosphoglucomutase

Two activity zones were detected for phosphoglucomutase. One of them, the more anodal one, was well characterized under the electrophoretic conditions used and was designated PGM1. The other was not well defined and therefore was excluded from the present analyses.

PGM1 was present in all phases of ontogenetic development and was more deeply staining during the larval and pupal stages. Phenotypic variants were detected for PGM1, suggesting that this enzyme is genetically controlled by a single locus with four codominant alleles: *PGM1*1*, *PGM1*2*, *PGM1*3*, and *PGM1*4*. Of the possible phenotypes resulting from the combination of these alleles, we did not detect PGM1 1, PGM1 4 or PGM1 1-4. Heterozygous individuals presented two bands of equal staining intensity (Figures 6A and 7).

Pashley *et al.* (1985) also detected a PGM zone in the populations studied by them, the *PGM* locus having five alleles in one and four alleles in the remaining populations.

Phosphoglucomutase has been extensively investigated. Studies conducted on several lepidopteran species have detected a single PGM locus with genetic variation, usually with more than three alleles (Lokki *et al.*, 1975; Brittnacher *et al.*, 1977; Sluss *et al.*, 1978; Harrison *et al.*, 1983; Pashley, 1983; Willhite and Stock, 1983; Pashley and Johnson, 1986; Brussard, 1989).

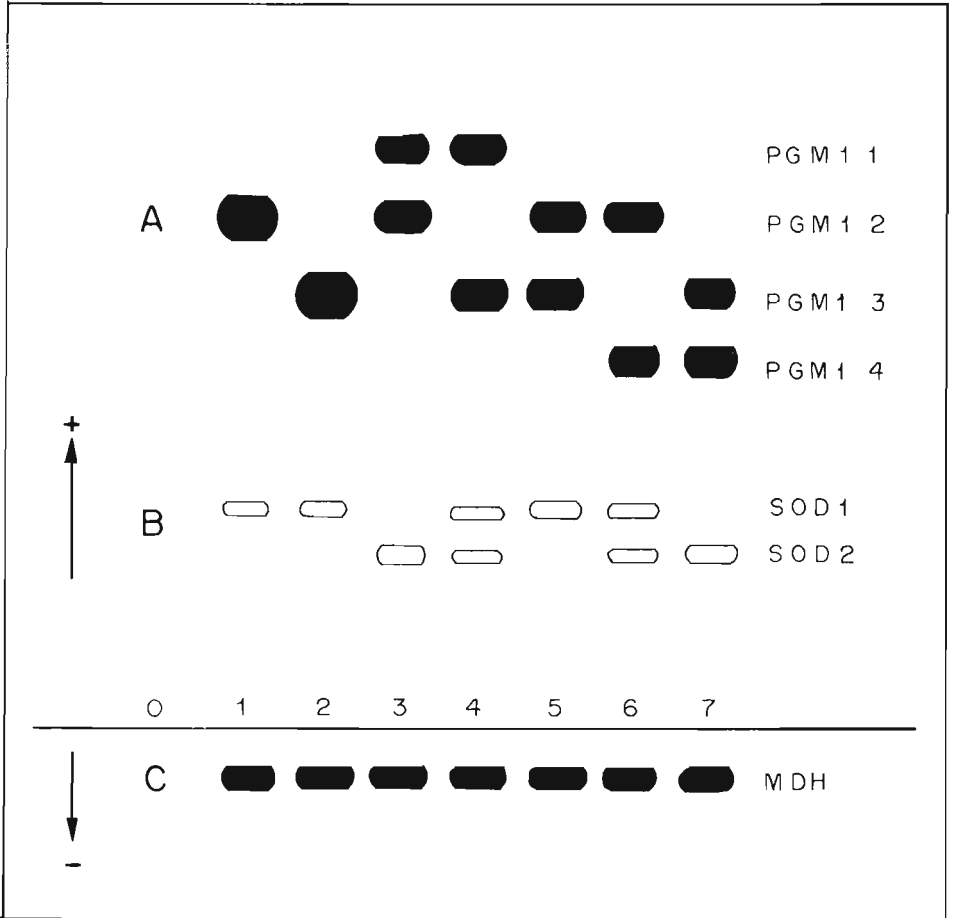


Figure 6 - Schematic presentation of the electrophoretic patterns obtained for extracts of *S. frugiperda* pupae. (A) Phenotypes detected for phosphoglucomutase. Sample 1, PGM1 2; sample 2, PGM1 3; sample 3, PGM1 1-2; sample 4, PGM1 1-3; sample 5, PGM1 2-3; sample 6, PGM1 2-4; sample 7, PGM1 3-4. (B) Phenotypes detected for superoxide dismutase. Samples 1, 2 and 5, SOD 1; samples 3 and 7, SOD 2; samples 4 and 6, SOD 1-2. (C) Malate dehydrogenase.

Superoxide dismutase

The electrophoretic pattern of *S. frugiperda* superoxide dismutase consisted of a single activity band of anodal migration. During ontogenetic development SOD is absent until the end of the larval stage, is present in prepupae though with weak

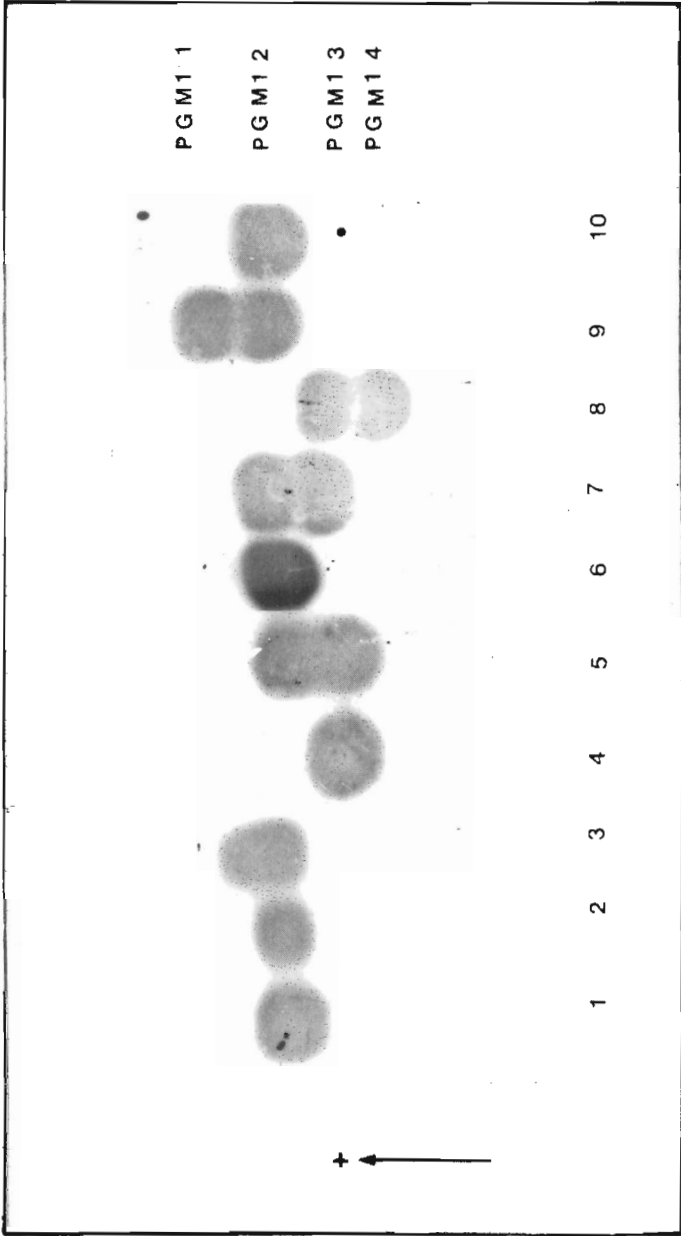


Figure 7 - Photograph of a gel stained for phosphoglucosyltransferase. Samples 1, 2, 6 and 10, PGM1 2; samples 3 and 9, PGM1 1-2; sample 4, PGM1 3; samples 5 and 7, PGM1 2-3; sample 8, PGM1 3-4.

staining intensity, shows stronger staining intensity in the pupal stage and is weaker again in the adult phase.

We detected phenotypic variations suggesting that the *SOD* coding locus of *S. frugiperda* has two codominant alleles *SOD*1* and *SOD*2*. Heterozygous individuals presented two bands of equal intensity. Schematic presentations of the phenotypes are illustrated in Figure 6B.

In other lepidopterans in which *SOD* was studied, two loci have been detected which are monomorphic in ten species of the genus *Speyeria* (Brittnacher *et al.*, 1977) and in *Lymantria dispar* (Harrison *et al.*, 1983) and polymorphic in *Euphydryas* spp. (Brussard, 1989). In *Solenobia triquetrella*, one locus is monomorphic and the other polymorphic (Lokki *et al.*, 1975). In *Heliothis virescens* and *H. zea* (Sluss *et al.*, 1978) two *SOD* loci serve as genetic markers to differentiate the two species since one of them is polymorphic in *H. virescens* and monomorphic in *H. zea* and vice-versa.

Malate dehydrogenase

The electrophoretic pattern of *S. frugiperda* malate dehydrogenase was quite simple, consisting of only one band of enzyme activity of cathodal migration. We believe that there may be only one locus coding for MDH and that this locus is monomorphic (Figure 6C).

In terms of ontogenetic development, our studies are incomplete, since we have no data about the egg stage and the first larval stages. MDH activity was detected from the 6th larval instar to the adult phase.

Studies carried out on other lepidopterans have identified two MDH loci, at least one of which is monomorphic in the species analyzed (Lokki *et al.*, 1975; Brittnacher *et al.*, 1977; Stock and Castroville, 1981; Willhite and Stock, 1983; Harrison *et al.*, 1983; Pashley, 1983; Dubach *et al.*, 1988).

Analysis of the data obtained in the present study permits us to conclude that starch gel and/or starch-agarose gel electrophoresis when used for protein fractionation together with specific staining proved is still a very useful technique for the determination of the electrophoretic pattern of each protein studied and of the number of loci involved and for the identification of the phenotypes resulting from allelic variants. Of the 12 proteins studied, six (ME, GDH, ADH, PT, SOD and MDH) are described for the first time for *S. frugiperda*. A total of 22 loci were detected, 13 of which presented genetically determined variation.

Population data related to allelic and genotypic frequencies, the comparison of genotype distribution between sexes, intralocus and mean heterozygosity, as well as comparative analysis of the two populations are currently being analysed.

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RESUMO

Spodoptera frugiperda (J.E. Smith, 1797) (Lepidoptera: Noctuidae) é considerada uma das maiores pragas da cultura de milho, arroz e outras gramíneas, sendo que seu controle é de grande importância econômica.

Os estudos realizados sobre essa espécie, têm levado em conta os danos causados à cultura e controle da praga. Trabalhos sobre variabilidade genética a nível proteico, dentro e entre populações de *S. frugiperda*, praticamente são inexistentes.

O presente trabalho tem como objetivo analisar doze proteínas: peptidases, carboxilesterases, enzima málica, glicose desidrogenase, álcool desidrogenase, glicerol-3-fosfato desidrogenase, isocitrato desidrogenase, leucina-aminopeptidase, proteínas não específicas, fosfoglicomutase, malato desidrogenase e superóxido dismutase, em populações naturais de *S. frugiperda* a fim de determinar o perfil eletroforético das mesmas, número de locos envolvidos e identificar possíveis variantes alélicas para as mesmas.

Das doze proteínas estudadas, seis delas (ME, GDH, ADH, PT, SOD e MDH) são descritas pela primeira vez. Foram detectados vinte e dois locos, sendo que treze apresentaram variação geneticamente determinada. A análise estatística referente aos dados populacionais está em preparação.

REFERENCES

- Brittnacher, J.G., Sims, S.R. and Ayala, F.J. (1977). Genetic differentiation between species of the genus *Speyeria* (Lepidoptera: Nymphalidae). *Evolution* 32: 199-210.
- Brussard, P.F. (1989). Complex population differentiation in checkerspot butterflies (*Euphydryas* spp). *Can. J. Zool.* 67: 330-335.
- Carvalho, R.P.L. (1970). Danos, flutuação da população, controle e comportamento de *Spodoptera frugiperda* (J.E. Smith, 1797) e susceptibilidade de diferentes genótipos de milho em condições de campo. Doctoral thesis, Escola Superior de Agricultura Luiz de Queiróz-USP, Piracicaba.
- Den Boer, M.H. (1978). Isoenzymes and migration in the African armyworm *Spodoptera exempta* (Lepidoptera, Noctuidae). *J. Zool.* 185: 539-553.

- Dubach, J.M., Richman, D.B. and Turner, R.B. (1988). Genetic and morphological variation among geographical populations of range caterpillar, *Hemileuca olivae* (Lepidoptera, Saturniidae). *Ann. Entomol. Soc. Am.* 81: 132-137.
- Harris, H. and Hopkinson, D.A. (1976). *Handbook of enzyme electrophoresis in human genetics*. North Holland.
- Harrison, R.G., Wintermeyer, S.F. and Odell, T.M. (1983). Patterns of genetic variation within and among gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) populations. *Ann. Entomol. Soc. Amer.* 76: 652-656.
- Lima, L.M.K.S. (1989). Variabilidade proteica em populações naturais de *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Doctoral thesis, Faculdade de Medicina de Ribeirão Preto-USP, Ribeirão Preto.
- Lokki, J., Suomalainen, E., Saura, A. and Lankinen, P. (1975). Genetic polymorphism and evolution in parthenogenetic animals. II, Diploid and polyploid *Solenobia triquetrella* (Lepidoptera: Psychidae). *Genetics* 79: 513-525.
- Luginbill, P. (1928). The fall armyworm. *Tech. Bull.* 34: 1-91.
- Menken, S.B.J. (1987). Is the extremely low heterozygosity level in *Yponomeuta rorellus* caused by bottlenecks? *Evolution* 41: 630-637.
- Pantoja, A., Smith, C.M. and Robinson, J.F. (1986). Effects of the fall armyworm (Lepidoptera: Noctuidae) on rice yields. *J. Econ. Entomol.* 79: 1324-1329.
- Pashley, D.P. (1983). Biosystematic study in Tortricidae (Lepidoptera) with a note on evolutionary rates of allozymes. *Ann. Entomol. Soc. Am.* 76: 139-148.
- Pashley, D.P. and Johnson, S.J. (1986). Genetic population structure of migratory moths: the velvetbean caterpillar (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 79: 26-30.
- Pashley, D.P., Johnson, S.J. and Sparks, A.N. (1985). Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 78: 765-762.
- Shows, T.B., Alper, C.A., Bootsma, D., Dorf, M., Douglas, T., Huisman, T., Kit, S., Klinger, H.P., Kozak, C., Lalley, P.A., Lindsley, D., McAlpine, P.J., McDougall, J.K., Meerakhan, P., Meisler, M., Morton, N.E., Opitz, J.M., Partridge, C.W., Payne, R., Roderick, T.H., Rubistein, P., Ruddle, F.H., Shaw, M., Spranger, J.W. and Weiss, K. (1979). Human gene mapping. Fifth International Workshop on Human Gene Mapping. *Cytogenet. Cell. Genet.* 25: 96-116.
- Sluss, T.P., Sluss, E.S., Graham, H.M. and Du Bois, M. (1978). Allozyme differences between *Heliothis virescens* and *H. zea*. *Ann. Entomol. Soc. Am.* 71: 191-195.
- Smithies, O. (1955). Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. *Biochem. J.* 61: 629-641.
- Sparks, A.N. (1979). A review of the biology of the fall armyworm. *Fla. Entomol.* 62: 82-87.
- Stock, M.W. and Castrovillo, P.J. (1981). Genetic relationships among representative populations of five *Choristoneura* species: *C. occidentalis*, *C. retiniana*, *C. biennis*, *C. lambertiana* and *C. fumiferana* (Lepidoptera: Tortricidae). *Can. Entomol.* 113: 857-865.

- Todd, E.L. and Poole, R.W. (1980). Keys and illustration for the armyworm moths of Noctuid genus *Spodoptera* Guenee from the Western Hemisphere. *Ann. Entomol. Soc. Am.* 73: 722-738.
- Willhite, E.A. and Stock, M.W. (1983). Genetic variation among western spruce budworm (*Choristoneura occidentalis*) (Lepidoptera: Tortricidae) outbreaks in Idaho and Montana. *Can. Entomol.* 115: 41-54.
- Wiseman, B.R., Painter, R.H. and Wasson, C.E. (1966). Detecting corn seedling differences in the greenhouse by visual classification of damage by the fall armyworm. *J. Econ. Entomol.* 59: 1211-1214.

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