evolved into a more aggressive form; or (iii) the insect was indigenous to Middle America but only recently transferred to *Gossypium* from a related host plant. There has been no positive evidence in support of any of these hypotheses. We now report observations that support the third hypothesis.

The genus *Hampea*, which has generally been regarded as a member of the family Bombacaceae, probably belongs in the Malvaceae (*5*) and is a close relative of *Gossypium*; with certain other genera, these form a natural tribe, the Gossypieae. Thus, species of *Hampea* become suspect as hosts of the boll weevil, especially in view of the geographical distribution of *Hampea*, which grows from western Colombia through Central America to southern Mexico, at least as far north as the states of Oaxaca and Veracruz. The other genera can be ruled out on the basis of available negative evidence concerning them or because of their geographical remoteness. The critical area seems to be in southern Mexico, where the ranges of *Hampea* and of *Gossypium* come in contact, and where, in fact, the first boll weevil was collected. In a gross geographical sense, the ranges overlap; however, *Hampea* generally grows in damp locations, while *Gossypium* prefers drier areas.

We undertook a field trip to this area in September 1966 to observe *Hampea* in its natural environment. Flowering trees of *Hampea*, tentatively determined to be *H. integerrima* Schlecht. (*6*), were found as a part of the natural vegetation in a number of areas in Veracruz. The species is dioecious, an unusual condition in the Malvaceae (although probably typical of the genus *Hampea*), and both male and female trees produce an abundance of small fragrant flowers in axillary clusters along the branches. The plants are known locally as "Majagua" and "Tecolistle." The former name, at least, is also applied to a number of other plants.

In one locality in Veracruz (between Martínez de la Torre and Misantla) all of the *Hampea* trees observed were heavily infested with boll weevils; most of the flower buds showed oviposition punctures. Apparently no cotton is cultivated at the present time within several hundred miles of the area where we collected the weevils. We found adult weevils on both male and female trees; more adults emerged from flower buds harvested from the male trees. The insects have been identified as *Anthonomus grandis* Boh. (*7*).

These facts suggest that *Hampea* is a natural host for *Anthonomus grandis*. It may, in fact, be the original host from which the insect transferred to *Gossypium* sometime during the 18th century when expanded cotton cultivation provided an opportunity for the insect to spread.

Standley, in the only published revision of the genus *Hampea* (*8*), recognized nine species; seven additional species have been published subsequently, and others probably will be discovered (*6*). Our observations from Veracruz are all that is yet known of the extent to which *Anthonomus grandis* occurs on *Hampea*.

Stands of *Hampea* were observed at the following localities in Veracruz: Papantla (collection number 522), Tecolapan (525), Lago Catemaco (526), between Catemaco and the Gulf coast (527), Martínez de la Torre (534), and between Martínez de la Torre and Misantla (535). Voucher specimens of plants from these localities, bearing the collection numbers given, will be deposited in the Tracy Herbarium (TAES) at Texas A&M University, and duplicates will be distributed elsewhere. These plants grew at elevations ranging from about 150 m for specimens 534 and 535 to about 1000 m for specimen 527. Only in the vicinity of Martínez de la Torre were weevils found infesting the *Hampea* trees. The weevil specimens collected will be placed in the collection of the Entomology Department of Texas A&M University.

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6. The specimens cited differ in some respects from typical *H. integerrima*, primarily in leaf form; they may be taxonomically distinct from it. The typical form has truncate elliptical leaves and occurs at relatively high elevations; the present form has cordate ovate leaves and is found at lower elevations. The plants differ clearly in floral characteristics from *H. rovirosae* Standl. from Tabasco.
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Acetylcholine Receptor: Similarity in Axons and Junctions

Abstract. *Sulfur and selenium isologs of benzoylcholine and its tertiary analog differ greatly in their abilities to block the electrical activity of squid axons. Presumably, differences in the biological activities of these compounds can be correlated with differences in their electron distribution. The relative effects on axons parallel those on junctions of the electroplax, suggesting the presence of similar receptors.*

Evidence has been accumulating supporting the assumption that acetylcholine (ACh) is essential for the control of the permeability cycle during electrical activity of all excitable membranes (*1*). While the essentiality of the components of the ACh system, for example acetylcholinesterase and the ACh-receptor, at junctional regions is generally accepted, their essentiality along axons has been disputed (*2*). The ACh theory of conduction along axons, as proposed by Nachmansohn, has found new support in the demonstration that while electrical activity of excitable membranes is reversibly blocked by potent competitive inhibitors of acetylcholinesterase (such as physostigmine), irreversible inhibitors, such as diisopropyl fluorophosphate (DFP), block activity irreversibly (see *1*).

The specificity of the latter effect was questioned because of the high concentration required, but it has been found recently that DFP is rapidly inactivated in axonal preparations by a phosphoryl phosphatase and that, therefore, the inhibitor enters only in low concentrations (*3*). Moreover, under proper conditions, 2-formyl-1-methyl pyridinium iodide oxime restores electrical activity blocked by organophosphates (*4*); this is of special interest since this compound specifically reactivates acetylcholinesterase inhibited by organophosphates by breaking the P-O bond formed during the phosphorylation (*5*). By a combination of electron microscopy and histochemical staining and by ultra-microgasometric methods, it has been shown that the enzyme is localized in or near the excitable membranes in axonal as well as in junctional membranes (*6*).

The evidence for the association of acetylcholinesterase and electrical activity was supplemented by evidence that electrical activity is also blocked by compounds that compete with

Table 1. Effect of oxygen (O), sulfur (S), and selenium (Se) isologs of the tertiary analog of benzoylcholine on the action potential of the squid giant axon. All compounds were applied for 30 minutes unless a block of electrical activity was obtained sooner. All recordings were made with extracellular electrodes. Number of experiments is indicated in parentheses. C, Control axons; V, axons treated for 30 minutes with 15 μg of cottonmouth-moccasin venom per milliliter. Results are presented as percentages of decreases of action potential (mean ± standard error). The effects of the Se-containing compounds were irreversible while all other effects were reversible. pH = 7.8; 18° to 21°C.

Substitution at:		Condition	$(CH_3)_2N-CH_2-CH_2-A-\overset{B}{\overset{\|}{C}}-C_6H_5$ (mole/liter)						
A	B		5×10^{-2}	1×10^{-2}	3×10^{-3}	1×10^{-3}	3×10^{-4}	1×10^{-4}	3×10^{-5}
O	O	C	100 ± 0 (2)	17 ± 2 (2)					
O	O	V		100 ± 0 (2)	70 ± 10 (2)				
S	O	C			100 ± 0 (2)	25 ± 5 (2)			
S	O	V				100 ± 0 (2)	40 ± 6 (3)		
Se	O	C				57 ± 10 (3)	20 ± 6 (3)		
Se	O	V					100 ± 0 (2)	10 ± 0 (2)	
O	S	C				100 ± 0 (3)	0 ± 0 (2)		
O	S	V					100 ± 0 (2)	50 ± 0 (2)	
S	S	C				100 ± 0 (2)	40 ± 10 (2)	0 ± 0 (2)	
S	S	V					100 ± 0 (2)	55 ± 5 (2)	
Se	S	C					100 ± 0 (4)	0 ± 0* (3)	0 ± 0* (2)
Se	S	V						95 ± 5 (4)	7 ± 7 (2)

*Repetitive or spontaneous firing at a rate of about 100 per second produced in 20 to 40 minutes. These continued for up to 1½ hours after return of axon to normal sea water. Concentrations greater than $1 \times 10^{-4}M$ or lower than $3 \times 10^{-5}M$ did not have this effect. Firing was not seen in axons treated with venom.

ACh for the receptor, such as local anesthetics analogous in structure to ACh. Analysis has shown which molecular features determine the transition from a receptor activator, such as ACh, into inhibitors having the ability to penetrate through structural barriers covering conducting membranes (*7*). Direct actions of ACh and curare on electrical activity of axons have been obtained. Curare reversibly blocks electrical activity of Ranvier nodes of single sciatic fibers from frogs, while ACh affects, and eventually blocks, electrical activity of axons from the walking-legs of lobster and from rabbit vagus (*8*). After squid axons are treated with snake venoms, ACh and curare block their electrical activity (*9*). The phospholipase A of these venoms disintegrates the Schwann cell, thereby reducing the permeability barrier. Radioactively labeled ACh, curare, and so on, unable to enter the axon before treatment, are found in the interior after the treatment.

A more refined analysis of the similarity of the ACh-receptor sites at junctions and in axons has been made possible by the synthesis of sulfur and selenium isologs of ACh, choline, and related compounds (*10*). The molecular sizes and shapes of sulfur and selenium isologs are very similar so that the ability of these isologs to fit active sites of a receptor should not be appreciably affected. In contrast, electron distribution in the stereoisomers may differ markedly, as indicated by kinetic, spectroscopic, and dipole measurements of isologous esters (*11*). Recently, 2-dimethlaminoethyl-benzoate, the tertiary analog of benzoylcholine, and analogs in which the side-chain oxygen had been replaced by sulfur and selenium, were tested on the synaptic junctions of an isolated electroplax preparation (*12*). As inhibitors of electrical activity, the O isologs were the weakest, the S isologs were stronger, and the Se isologs were most potent. Since these compounds are chemically closely related to ACh, one would expect that their effects are due to inter-

Table 2. Effect of oxygen (O), sulfur (S), and selenium (Se) isologs of benzoylcholine on the action potential of the squid giant axon. Experimental conditions described in Table 1. The effects of the compounds containing Se were irreversible, while all other effects were reversible. Results are given as percentages of decreases of action potential (mean ± standard error). C, Control axons; V, axons treated with venom.

Substitution at:		Condition	$(CH_3)_2N^+-CH_2-CH_2-A-\overset{B}{\overset{\|}{C}}-C_6H_5$ (mole/liter)					
A	B		9×10^{-2}	3×10^{-2}	1×10^{-2}	3×10^{-3}	1×10^{-3}	3×10^{-4}
O	O	C	20 ± 0 (2)	0 ± 0 (3)				
O	O	V	60 ± 2 (2)	27 ± 2 (2)	0 ± 0 (2)			
S	O	C		0 ± 0 (2)				
S	O	V		20 ± 0 (2)	0 ± 0 (2)			
Se	O	C			0 ± 0 (3)			
Se	O	V			100 ± 0 (4)	0 ± 0 (2)		
O	S	C			5 ± 5 (2)			
O	S	V			40 ± 10 (3)	0 ± 0 (2)		
S	S	C			5 ± 5 (2)			
S	S	V			100 ± 0 (2)	90 ± 10 (2)	0 ± 0 (2)	
Se	S	C			100 (1)	30* (1)	0 ± 0* (2)	0 ± 0 (2)
Se	S	V				100 ± 0 (2)	53 ± 24 (3)	5 ± 5 (2)

* Repetitive firing at a rate of about 100 per second produced in less than 10 minutes. These rapidly stopped upon return of axon to normal sea water. Concentrations greater than $3 \times 10^{-3}M$ or lower than $1 \times 10^{-3}M$ did not have this effect. Firing was not seen in axons pretreated with venom.

action with the ACh-receptor at the junction of this preparation. If the action of ACh is due to its effect on a specific receptor protein, one would expect that the active site of this protein is similar in all excitable membranes. If this is the case, O, S, and Se isologs should exhibit relative differences of potency in axons similar to those observed at junctions, thus indicating the similarity of the macromolecules reacting with these isologs and their essentially similar functions.

The effects of some O, S, and Se isologs of the tertiary analog of benzoylcholine on the action potential of squid giant axons have been recently reported (*13*). We have extended these studies (Table 1), and isologs of benzoylcholine, in which the carbonyl oxygen has been replaced by sulfur (*14*), have been included (Table 2). Substitution of O by S and further substitution by Se in a series of isologs of the tertiary analog of benzoylcholine results in a progressive increase of their potency (Table 1). At the *p*H of these experiments, the compounds are partly in their charged and partly in their uncharged form. The charged form would not be expected to readily penetrate the squid-axon membrane (*1*, *9*, *15*), while even the uncharged molecule might not be completely permeable. Partial disruption of the permeability barriers by treatment of the axon with cottonmouth-moccasin venom increased the potency of these isologs (Table 1). This concentration of venom has no effect on the height of the conducted action potential (*9*). The two isologs containing Se caused an irreversible block of conduction, whereas the effects of the other compounds were reversible. In view of the very high tendency of selenolesters, compared to thiolesters, to acylate certain nucleophiles (*16*), selenolesters related to ACh might prove useful tools for labeling the receptor protein. The last compound listed in Table 1 (Se, S) is of special interest since it produced repetitive or spontaneous firing, which continued for up to 1½ hours after the axon was returned to normal sea water. This compound might be especially suitable for analysis of drug-binding to and of induced activation of the ACh-receptor.

The quaternary analogs of the compounds shown in Table 1 were also tested (Table 2). Because of their lipid insolubility, these compounds would not be expected to penetrate readily to the membrane of the squid axon. Five out of the six compounds were inactive on control squid axons (Table 2), whereas after the axons are treated with venom their order of potency is similar to that previously shown for the tertiary compounds (Table 1), although the concentrations required are greater. The isolog containing Se and S was most potent and caused repetitive firing. There is at least a hundredfold range in potency in the series of compounds tested (Tables 1 and 2). It appears that, although these compounds are isosteric, they differ markedly in their abilities to bind to receptor sites or to induce conformational changes of the ACh-receptor, which, in turn, may be related to differences in their electronic distribution. Similarly, the pharmacological effects are greatly modified when the ether oxygen of acetylcholine is replaced by S or Se (*17*). Our results are in almost exact agreement with observations made on the junction of the isolated electroplax, where potency also increased as O was substituted by S and Se (*12*).

Our observations support the assumption that the specific sites of reaction of the ACh-receptor are similar in both axons and junctions, since subtle change of electron distribution of a molecule, without a change of its shape, induces similar modification of the biological action in both excitable membranes.

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Single Fibers of Cat Optic Nerve: "Thresholds" to Light

Abstract. *Absolute thresholds of 39 single fibers of the optic nerves of 20 cats were determined by inspection of post-stimulus time histograms, each computed from the responses to 60 to 100 identical flashes of white light. The values found—from 1.1 × 10^{-7} to 6.8 × 10^{-6} candella per square meter (nits)—agree well with psychophysical thresholds found in previous investigations.*

Preparations tested electrophysiologically appear to be considerably less sensitive than man and animals tested by behavioral methods (*1*). This discrepancy has led to investigations, with microelectrodes, of the absolute threshold in visual neurons of the cat (*2*, *3*). Results of these studies are about one log unit higher than the mean threshold found by behavioral methods (*4*, *5*). These discrepancies could be due to the nonphysiological state of the preparations, or to insufficient sensitivity of the data-processing techniques used to